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GENETIC AND PHENOTYPIC CATALOG OF NATIVE RESIDENT TROUT OF THE INTERIOR COLUMBIA RIVER BASIN

Fiscal Year 1998 Report: Populations of the Upper Yakima Basin

Annual Report 1999

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Fiscal Year 1998 Report:

Populations of the Upper Yakima Basin

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GENETIC AND PHENOTYPE CATALOG OF NATIVE

RESIDENT TROUT OF THE INTERIOR COLUMBIA RIVER BASIN

FY-98 REPORT: POPULATIONS OF THE UPPER YAKIMA BASIN

EXECUTIVE SUMMARY

In fiscal year 1998, we collected nonlethal fin tissues for genetic analysis from eleven stream trout populations (ten cutthroat populations and one rainbow population) residing in headwater reaches of of Yakima basin tributaries. Using a portable aquarium, we also photographed representative specimens of each population for a color catalog of appearance phenotypes. Analysis of paired interspersed nuclear DNA elements (PINEs) was used to characterize each population as to subspecies and level of hybridization, and a genetic purity rating was assigned to each using a modification of the Binns system originally developed in Wyoming to gauge the genetic purity of interior cutthroat trout populations.

Five of the cutthroat trout populations we collected were genetically pure westslope cutthroattrout *Oncorhynchus clarki lewisi*. The other five populations were good examples of *lewisi* aswell, appearance-wise, but these populations contained individuals hybridized with rainbow trout. The percent of hybrid individuals in these populations ranged from less than 5 percent to 20 percent. Owing to a limitation of the PINE technique, we were unable to state whether the rainbow trout contribution to the hybrids was from the Columbia River redband subspecies*O. mykiss gairdneri*, which occurs in the lower reaches of most if not all of the same stream systems, or from the coastal rainbow subspecies *O. M. irideus* which has been widely stocked in the basin. In terms of the Binns purity rating, only two of the five genetically pure *lewisi* populations were given A–ratings (no hybridization and no history of cutthroat trout stocking anywhere in the system). The other three pure *lewisi* populations were rated B because they occur in streams where hatchery cutthroat trout have been stocked in the past.

The single rainbow trout population examined in this study was interesting in that it was a mixture of subspecies, 75 percent of the individuals being the Columbia River redband subspecies and 25

percent being coastal rainbow trout. No hybrid individuals were found. The presence of coastal rainbow trout suggests a hatchery-stocking influence even though no official stocking of any kind has occurred in this stream since 1973. However, as none of the fish we collected were hybrids, regardless of how the coastal rainbow got there, reproductive isolation has evidently been maintained in this stream.

Our discovery of two A–populations (no hybrids and no history of stocking) of westslope cutthroat trout in headwater tributaries of the Yakima basin lends additional weight to earlier suggestions that the range of *lewisi* extends into central Washington State westward to the Cascade crest. It also suggests that the evolutionarily younger and later-invading redband rainbow trout has not completely displaced native cutthroat from this range, especially not from the uppermost reaches of trout-bearing waters.

The precise locations of our collection sites as well as site descriptions, site photographs, and habitat conditions as we found them are given in the report. Maps showing the distribution of genetically pure and hybridized populations within the Yakima basin are also included. Although not a complete inventory by any means, this information should be of great value to managers in coming years for stewardship of the native resident trout populations, especially in the face of the potentialial listing of the westslope cutthroat trout under the U. S. Endangered Species Act.

INTRODUCTION

The 1994 Fish and Wildlife Program of the Northwest Power Planning Council specifies the recovery and preservation of population health of native resident fish of the Columbia River Basin. Among the native resident species of concern are interior rainbow trout of the Columbia River redband subspecies *Oncorhynchus mykiss gairdneri*[1] and westslope cutthroat trout *O. clarki lewisi*. The westslope cutthroat trout has been petitioned for listing under the U. S. Endangered Species Act (American Wildlands et al. 1997).

[1] The common and scientific names used here are those of Behnke (1992).

Before at-risk populations can be protected, their presence and status must be established. Where introgression from introduced species is a concern, as in the case of both westslope cutthroat trout and redband rainbow trout, genetic issues must be addressed as well. As is true with native trout elsewhere in the western United States (Behnke 1992), most of the remaining pure populations of these species in the Columbia River Basin are in relatively remote headwater reaches.

The objective of this project is to photo-document upper Columbia Basin native resident trout populations in Washington, and to ascertain their species or subspecies identity and relative genetic purity using a nonlethal DNA technique. The overall project will extend over five years, with the intent being to conduct field visits to remote locations to seek out these populations. This project will complement a similar BPA-funded project initiated by the Nez Perce Tribe to catalog the genetic purity of westslope cutthroat trout populations in the Clearwater River basin (Weigel 1997; Spruell et al. 1997)).

At the urging of several stakeholder groups in the Columbia Basin Resident Fish and Wildlife Program, we devoted our FY-1998 field work to the Yakima River basin.

THE STUDY AREA

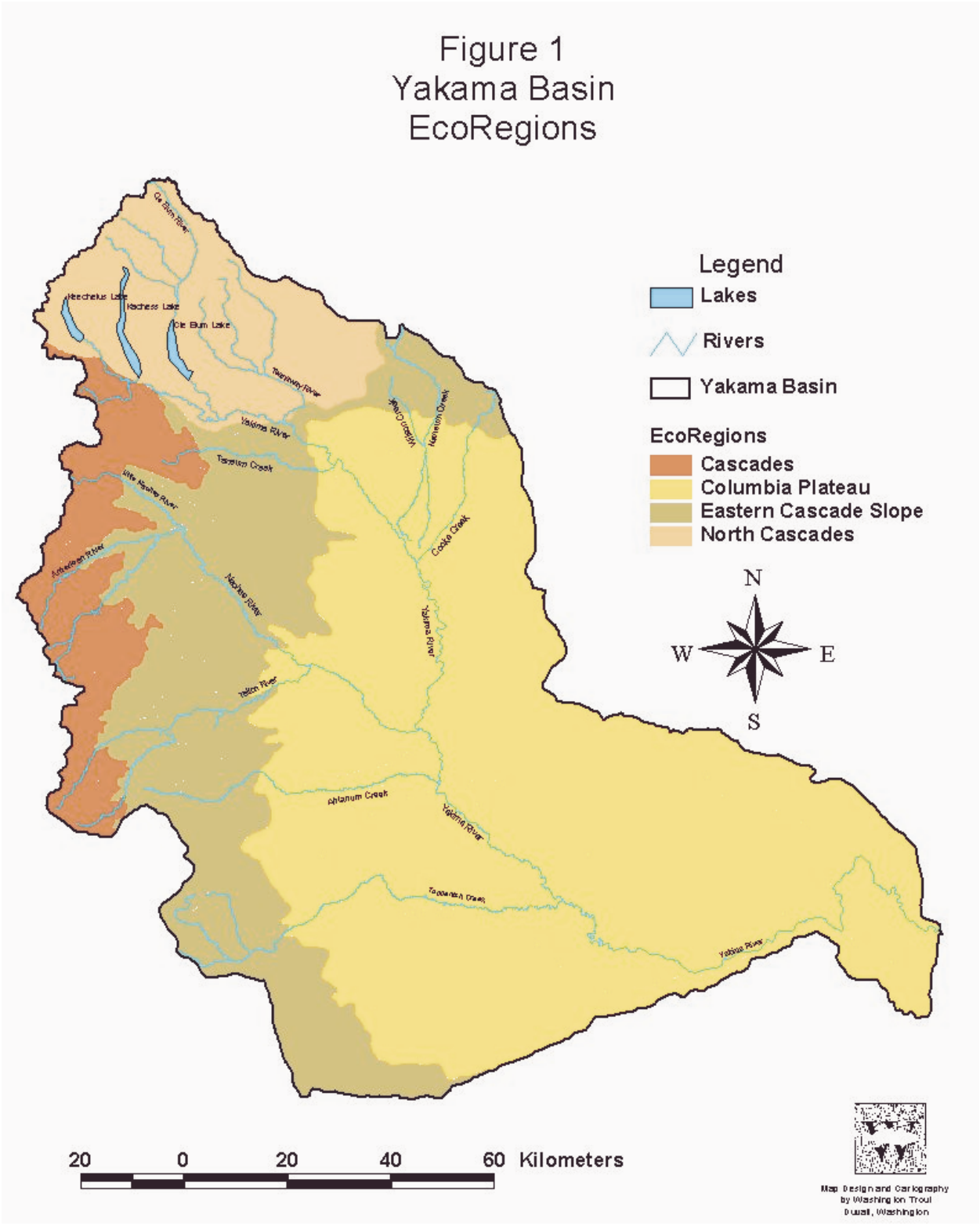
Yakima River Basin

The Yakima River and its tributaries drain an area of 15,942 km^2 (6,154 square miles) on the eastern slopes of the central Cascade Mountains east of Seattle, Washington. The basin extends from the Cascade crest on the west to the Columbia basin on the east, and from the Wenatchee Mountains on the north to the crest of the Horse Heaven Hills on the south. The main Yakima River flows about 344 km (214 miles) southeast from its headwaters to its confluence with the Columbia River near Richland. The Naches River, the largest tributary, joins the Yakima River near the City of Yakima.

The Yakima Basin lies across four defined ecoregions: 1) the North Cascades, 2) Cascades, 3) Eastern Cascades Slopes and Foothills, and 4) Columbia Plateau ecoregions (Pater et al. 1998; Figure 1). Ecoregions represent unique combinations of landscape features having distinctive terrestrial vegetation and climate (Omernik 1987). Terrain in the North Cascades and Cascades portions of the basin is forested and rugged with the average elevation along the Cascade crest about 2,100 m (6,900 ft). Principal land uses are forestry and recreation. Much of the basin within the Eastern Cascades Slopes and Foothills ecoregion is forested as well, with principal land uses being silvaculture and grazing. Shrub-steppe habitat is the natural state in the Columbia Plateau portions of the basin lying to the east and southeast, but much of this area is now in irrigated agriculture. Basin climate ranges from cool and moist (precipitation over 254 cm (100 inches) annually) in the mountains to warm and dry (precipitation about 20.3 cm (8 inches) annually) on the Columbia Plateau.

Major upper-basin tributaries of the Yakima River include Meadow Creek, which flows into Keechelus Reservoir (from which the Yakima River emerges), Cabin Creek, Big Creek, and the Kachess and Cle Elum rivers. Downstream, near the town of Cle Elum, the Yakima enters a canyon area. Important tributaries in this area are the Teanaway River and Swauk Creek. Further downstream, near Ellensburg, the river enters the Kittitas Valley, an area dominated by cattle grazing and hay production. Taneum, Manashtash, Reecer, Wilson, Naneum creeks, the Cooke Coleman system, and Cherry Creek are important tributaries here. Each of these tributaries is used

Figure 1
Yakama Basin
EcoRegions



for irrigation, both as a source of water and for irrigation return. South of Ellensburg, the Yakima River enters another canyon that is heavily utilized by recreational boaters and anglers. This reach of river is renown for its rainbow trout fishery. Umptanum Creek enters the Yakima in this canyon reach. The lower valley, generally from the City of Yakima downstream to the Columbia River confluence, is nationally known for its fruit production and vineyards, and in addition, grows many other crops such as hops, mint, and asparagus. Ahtanum Creek, Toppenish Creek, and Satus Creek, which head up in the high, forested terrain of the Cascades (Ahtanum Creek) and Eastern Cascades Slopes and Foothills ecoregions (Toppenish and Satus creeks), are major tributaries of the Yakima River in the lower valley.

The Yakima River and its tributaries support many water-related uses, including irrigation, hydropower generation, and drinking water (about 60 percent of the mean annual streamflow from the basin is diverted for these three purposes) as well as fish and wildlife, aquatic and riparian ecosystems, and recreation (BuRec 1998). It is one of the most intensively irrigated areas in the United States, with close to 2500 km^2 (965 sq. mi.) under irrigation (Cuffney et al. 1997; BuRec 1998). Downstream from the City of Yakima, during the irrigation season, return flow from irrigation comprises 80 to 90 percent of the flow in the lower mainstem (Fuhrer et al. 1996). One effect of this heavy irrigation is to have essentially isolated the headwater areas of many streams in the basin.

In pre-development time, hundreds of thousands of adult salmon and steelhead returned to the Yakima River system each year (McIntosh et al. 1994). By 1920, the total anadromous return had been reduced to about 11,000 fish per year, and in 1981, a low of just 2000 anadromous adults returned to the basin (NWPPC 1989; BuRec 1998). Coho salmon *Oncorhynchus kisutch* have become extinct in the basin, although attempts are being made to reintroduce this species via fry plants of stocks from elsewhere in the Columbia River system (David Lind, Yakama Indian Nation, personal communication 1998).

METHODS

Selection of Collection Sites

We compiled a list of Yakima Basin streams where resident populations of trout have been reported, using two general sources of information: 1) agency reports and personal communications from the Washington Department of Fish and Wildlife, Washington Department of Natural Resources, Yakama Indian Nation, U. S. Forest Service, and U. S. Fish and Wildlife Service; and 2) other sources including Bryant and Parkhurst (1950), the University of Washington Fish Collection, old fishing guidebooks, and anecdotal reports from longtime anglers. We then winnowed the list down based on the following criteria:

- Broad coverage of the upper basin with a reasonable selection of sites across ecoregions.

- No lakes in the headwaters of the drainage.

- All sites in headwater reaches upstream of any known influence of anadromous salmonids.

The no-lakes criterion was included to avoid at least one possible source of hatchery origin fish. The Washington Department of Fish and Wildlife propagates the Twin Lakes strain of westslope cutthroat trout and stocks these fish widely in high lakes of the Cascades and North Cascades ecoregions (Crawford 1979). Even though great pains are taken not to stock lakes where the fish might escape to reproduce downstream, such escapes have been known to occur nevertheless, so we generally avoided streams with lakes in their headwaters. We relaxed this criterion for two site selections: 1) Red Rock Creek, a tributary of the upper Bumping River, because it is near an area that could be impacted by a Bureau of Reclamation proposal to raise Bumping Lake Dam (a proposal that Washington Trout is monitoring closely); and 2) Meadow Creek, an easy to reach tributary of Keechelus Lake at the headwaters of the Yakima River. Meadow Creek has a robust trout population, and was a good site to round out our collections at the end of the field season. Both streams have small lakes in their headwaters.

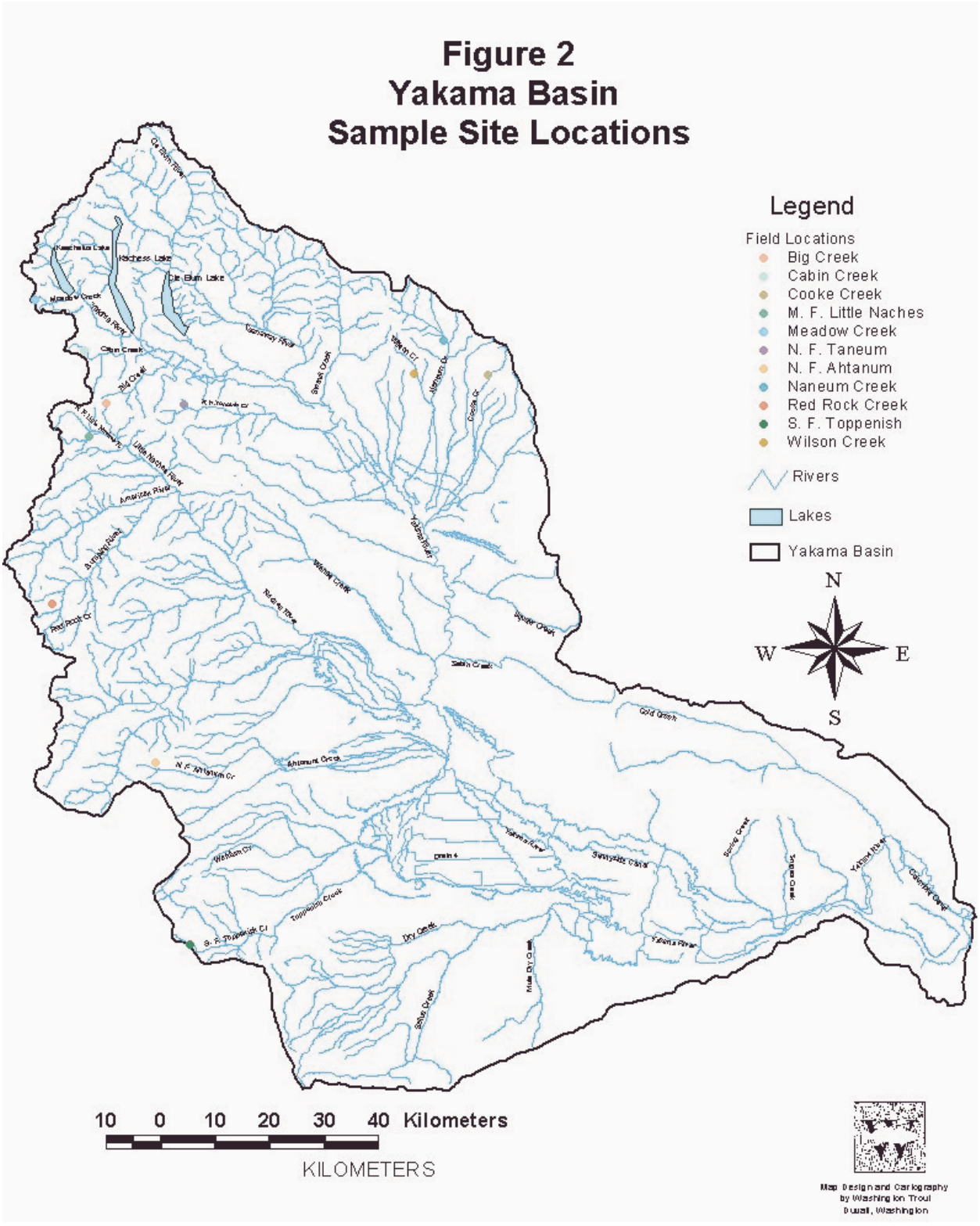
We later learned that we had inadvertently allowed one exception to the thrid criterion as well, that being the Middle Fork Little Naches River, which is indeed accessible to anadromous salmonids in the reach where we collected.

The final list included ten streams said to contain cutthroat trout populations and one stream, Cooke Creek, reported to contain a population of interior rainbow trout of the Columbia River redband subspecies (G. McMichael, Washington Department of Fish and Wildlife, Ellensburg, Washington, personal communication 1998). Cooke Creek lies immediately east of a stream which had been reported to be inhabited by cutthroat trout and could, thus, represent a range boundary between the two species within the basin. Seven of the collection sites are tributaries of the upper Yakima River between the east slope of Snoqualmie Pass and Kittitas Valley; two sites are tributaries of the Naches River; and two sites are tributaries to the lower Yakima River downstream of the confluence of the Naches. Site elevations and other vital statistics are given in Table 1, and site locations are mapped in Figure 2.

Table 1. Stream Reach Data

Stream Name	Map Coordinates	GPS Coordinates	Reach Altitude	Stream Order	Reach Gradient	Water Temperature	Habitat Score
N. Fk. Taneum Creek	19N 14E 21W	N47 07.50' W121 03.91'	1213m	2	0.04	7.8 C-NA	337
Cabin Creek	20N 12E 23W	N47 12.82' W121 18.41	927m	2	0.03-0.05	10.8C - 13.9C	207
Naneum Creek	20N 19E 15W	N47 13.24' W120 26.33	1036m	3	0.04	12.5C - 13.3C	233
Wilson Creek	20N 18E 36W	N47 08.05' W120 30.55	986	2	0.06	8.9C - 12.8C	211
Big Creek	19N 13E 8W	N47 08.05' W121 14.99	1049m	2	0.04	6.7C - 7.8C	257
S Fk Toppenish Creek	9N 14E 28W	N46 13.83' W121 04.89'	1061m	2	0.025	10.3C - 14.4C	222
N Fk. Ahtanum Creek	12N 14E 14W	N46 31.82' W121 09.51'	1426m	3	0.06	8.1C - 12.2C	249
Cooke Creek	19N 20E 9W	N47 09.39' W120 20.52	1061m	3	0.05	14.4C - 15.8C	194
M Fk. Little Naches R	19N 12E 36W	N47 05.11' W121 17.84	1036m	3	0.03	9.2C - 11.1C	260
Red Rock Creek	15N 11E 14W	N46 47.76 W121 23.65	1122m	2	0.016	7.8C - 10.0C	291
Meadow Creek (A)	21N 11E 15W	N47 18.31' W121 23.17	878m	3	NA	9.7C	226
Meadow Creek (B)	21N 11E 16W	NA	1000m	2	NA	NA	NA

Figure 2
Yakama Basin
Sample Site Locations



Stocking History

Since 1932, when the Washington legislature vested all responsibility for fish and wildlife management in what is now the Washington Department of Fish and Wildlife, the stocking of hatchery-reared trout has been a bread-and-butter practice of that agency. Prior to 1932, many other agencies and entities, including the State, the old U. S. Bureau of Fisheries, county fish and game agencies, and even individuals, also stocked trout in state waters. Unfortunately, no neat institutional history exists for any of these activities. We canvassed the archives of the Washington Department of Fish and Wildlife, all of the old State Fish Commissioner's reports that we could find (Washington State Fish Commissioner 1905, 1907…through 1919), and any other sources that came to our attention (e.g., Varley 1979) for whatever records that might exist of trout-stocking activities in our collection streams and nearby waters. Although we cannot vouch for the completeness of these archives—and thus, can never be completely certain that the absence of a record for a given stream means no stocking ever occurred there—we nevertheless took the absence of a record as evidence that the population we found was native and untainted by stocking unless our genetic results indicated otherwise.

Based on old records and reports, hatchery origin or non-native fish likely to be encountered in the course of this investigation include coastal rainbow trout *Oncorhynchus mykiss irideus* (according to Crawford 1979, all of Washington's hatchery rainbow broodstocks are derived from *irideus*), coastal cutthroat trout *O. clarki clarki*, Yellowstone cutthroat trout *O. clarki bouvieri* (imported into the state in the past under the name "Montana black-spotted trout") and the Twin Lakes strain of westslope cutthroat trout mentioned above. Past shipments of "Montana black-spotted trout" into the state may also have included westslope cutthroat trout.

Other Recent Genetic Studies in the Basin

A review of the fisheries literature turned up three additional genetic studies of resident trout populations in the Yakima Basin. The first, by Campton and Johnston (1985), was a protein electrophoresis examination of resident rainbow trout from three mainstem sites and the lower reaches of two tributaries, all upstream of Ellensburg. No cutthroat trout populations were examined. The second, by Phelps (1993), was also a protein electrophoresis examination of resident

rainbow trout conducted by the Washington Department of Fish and Wildlife as part of the Yakima Species Interaction Study. This particular study focused on rainbow trout populations inhabiting the mainstem Yakima River and the lower reaches of several tributaries accessible to anadromous fish. One cutthroat trout population (from Wilson Creek, same location as our collection site) was collected for use as an out-group. The third study was a more wide-ranging one that included Wenatchee basin sites as well as sites in the Yakima basin (Ringel 1996, 1997; Proebstel 1998). This study, conducted for the U. S. Fish and Wildlife Service, examined both rainbow and cutthroat trout populations using several analytical techniques, with collections sometimes being made from the lower, middle, and upper reaches respectively of the same stream. We reviewed the results of each of these studies and incorporated peritnent findings in this report.

Fish Collection and Work-Up

In the field, upon arrival at a collection stream, we first prospected for a convenient work-up site where we could set up our aquarium and other gear. We recorded the township, range, and section coordinates of this site from the appropriate USGS 7-1/2' quadrangle map, and also its GPS coordinates read from a Garmin II-Plus unit. We also photographed the site and recorded its altitude and stream order (Strahler 1957) as determined from the map.

When our equipment was set up, we deployed upstream and down from the work-up site to collect fish. We seldom had to cover more than 1.2 km (3/4 mile) of stream to collect all the fish we needed; however, on one occasion we were obliged to range farther than that. On that occasion, we picked a second site on the stream, established its coordinates, and filled out our collection from that second site. We assigned each stream and collection site its own letter-code for identification.

We collected all fish specimens by hook and line angling using flies with barbless hooks. When a fish was brought to hand, we removed the fly and quickly placed the fish in a bucket of clean stream water, which itself was kept in the shade. We exchanged the holding water frequently to keep the fish cool and well-aerated. After 30-45 minutes of angling, we brought the fish to the work-up site, regardless of how many had been captured. If more fish were needed to complete our collection after the initial batch had been processed and released, additional 30-45 minute angling periods were

employed. We recorded the total time spent angling to capture the requisite number of fish from each site and used that as a rough index of fish abundance for the site.

Fish were anesthesized in groups of two or three, using the procedure described below. Each fish was then measured (fork length to the nearest mm) on a measuring board, weighed (wet weight to the nearest gram) using calibrated Pesola precision spring scales, and the adipose fin (or, on fish smaller than about 76 mm, a small snippet from the lower tip of the caudal fin) was removed with sharp, clean, stainless-steel scissors. These fin-tissue snippets were carefully placed in individual pre-labeled vials of preservative and saved for later use in the genetic analysis. The fish were then placed either in a bucket of clean stream water, or in a still but not stagnant part of the stream itself to recover from the anesthetic prior to release.

Anesthetic Protocol

We used clove oil (Anderson et al. 1997; Preiser et al. 1997) as our anesthetic in this work, after first running a comparison test against MS-222 (Argent Chemicals "Finquel"). We found that, as reported, clove oil produces the same levels of anesthesia on about the same timetable, and recovery times are also about the same, as MS-222 at equal concentrations. Plus, clove oil carries a GRAS (Generally Recognized As Safe) rating from the federal Food and Drug Administration whereas MS-222 must be used with a 21-day withdrawl period before the fish can become fodder for human consumption. This can be an important consideration when collecting from streams open to recreational angling, as most of our sites were. We started out using clove oil at 100 mg/L, but quickly backed off to 50 mg/L for our field work. At the 50 mg/L level, anesthesia was induced in 90 seconds to two minutes, and recovery of equilibrium generally occurred in about five to seven minutes.

Clove oil is not completely soluble in water, and must first be dissolved in ethanol. We prepared stock solutions consisting of 3 mL of clove oil (density approximately 1 g/mL) made up to 30 mL with denatured 95-percent ethanol. Three-mL quantities of this stock solution were measured out into individual ethanol-proof capped vials which were kept in the dark in a refrigerator until taken into the field. The contents of one vial dispersed in 6 L of stream water in a 18.9 L (5 gal.) bucket gave us our 50 mg/L field concentration.

Calculation of Condition Index

Condition indices are widely used to assess robustness or physiological well-being of fishes. Fulton Condition Factor, **K**, obtained by dividing the weight of the fish by the cube of its fork length, has been perhaps the most-used index of fish condition (Ricker 1975), but has been criticised for having inherent length-related and species-related biases (Cone 1989; Murphy et al. 1991). Therefore, we chose to calculate Relative Weight, $\mathbf{W_r}$, first proposed as an index of fish condition by Wege and Anderson (1978), for each of our specimens. $\mathbf{W_r}$ is given by the formula:

$$\mathbf{W_r = (W/W_s) \times 100} \qquad (1)$$

where **W** is the weight of each individual fish (in grams) and $\mathbf{W_s}$ is a length-specific standard weight which is computed from one of these equations (Kruse and Hubert 1997; Simkins and Hubert 1996):

For interior cutthroat trout $$\mathbf{\log_{10} W_s = -5.139 + 3.072 \log_{10} TL} \qquad (2)$$

For rainbow trout $$\mathbf{\log_{10} W_s = -5.023 + 3.024 \log_{10} TL} \qquad (3)$$

where **TL** is total length (in mm).

In equations (2) and (3), length is specified as total length, **TL**. Since we recorded fork length, **FL**, in the field, we converted using these formulae (Carlander 1969):

For interior cutthroat trout $$\mathbf{TL = 1.050\ FL} \qquad (4)$$

For rainbow trout $$\mathbf{TL = 1.071\ FL} \qquad (5)$$

Thus, to compute $\mathbf{W_r}$ from our field data, we applied equations (2) and (4) to the cutthroat trout collections and equations (3) and (5) to the rainbow trout collection to first compute $\mathbf{W_s}$ for each fish, then we plugged those values into equation (1) to compute the respective $\mathbf{W_r}$ values.

Statistical Analysis

Several statistical procedures were run on the collected data to screen our sampling methods for bias and to test for differences in mean condition index among sampled populations. Statistical analyses were performed using the NCSS 2000 statistical software package (Hintze 1999).

Because the Relative Weight index is dimensionless and is expressed as a proportion or percentage (= proportion *100), the W_r's cannot be expected to be normally distributed, nor can the variances of the samples be expected to be equal. W_r expresses fish condition independent of fish size (weight, length); for example, resident westslope cutthroat of 120mm and 250mm fork-lengths may both have a W_r of 90 (0.90). Hence, even if the original length distributions are normal and all sample populations have equal variances, one should expect the W_r's of a randomly sampled local population to have a repulsed distribution, with more individual values clustered close to the population mean value than in a normal distribution. The distributions of sample proportions can often be rendered normal by arcsine transformation. However, since W_r can have values greater than 100 (proportion greater than 1.0), arcsine transformation could not be used to attempt to normalize the repulsed data or equalize the sample variances. This generally invalidates the use of Analysis of Variance, which strictly requires that the sample populations to be analyzed have normal distributions and that their variances be equal.

Nevertheless, several Anovas were run on the W_r data to compare sample means, since the F-test is robust to mild violations of the normality and equality of variance requirements, provided the groups analyzed are random samples from their respective populations. Viewed in conjunction with Box Plots of the W_r data (Figure 5) and Anovas on the length data of the samples (see below), and with the above caveats in mind, the analyses present a fairly reliable picture of the data.

The following tests were run on the W_r data. A One-Way Analysis of Variance on sample means was run to test for difference among the mean relative weights of the sampled populations. Because our collections spanned the entire summer season we also conducted a Nested Anova using the NCSS GLM Anova tool with bi-weekly period of sampling the fixed factor and stream

sample population the nested factor to test for the influence of collection date on differences in sample populations mean W_r values.

Length data from the sampled populations provides a better indication of the reliability (randomness) of our sampling methods than W_r data. The length data of each sample was screened for normality using the NCSS Descriptive Statistics tool, and One-Way and Nested Anovas were run on the length data as they were for the relative weight data.

We also tested whether W_r values were correlated with length, since a strong correlation would indicate bias in the standard weight formula (W_s), bias in sampling method, or an unexpected length-related causal condition. Least squares regressions were run on each sampled population with individual W_r values as the dependent variable and individual length values the independent variable and scatter plots with the least square regression line through them were produced and inspected. Under ideal conditions and random sampling, there should be no correlation between W_r and length, and the slope of the regression line should be zero.

Finally, even though our collection site habitat quality scores (see below) were only qualitative, we tested for the relationship between population mean condition index and collection site habitat quality with linear (least squares) regression and scatter plots, as we did for W_r and length.

Fish Photography

Four to six fish (most often six) from each collection site were not anesthesized immediately, but were placed individually in a small portable aquarium through which stream water was flowing, allowed time to acclimatize, and then photographed. Following its photo session, each fish was removed from the aquarium, anesthesized, and worked up as described above while the next fish was becoming acclimatized to the aquarium.

The aquarium used in this work is a portable “photarium” unit of the type described by Rinne and Jakle (1981) and built according to their specifications. The unit is made of Plexiglas and measures

356 mm (14 inches) wide by 203 mm (8 inches) high by 51 mm (2 inches) deep. A small submersible pump (Teel model 1P811A) powered by a 12-volt gel-cell battery circulates stream water through the unit, thus maintaining an environment similar in temperature and oxygen content to the fish's natural habitat. The current in the tank induces the fish to assume a natural position without undue stress, enabling high quality color photographs to be obtained that are useful for documentation, taxonomic studies, and publication.

Much prior experience photographing fish in this portable aquarium dictated how we used the unit in the present work. Direct natural lighting was used, with the light impinging on the front of the aquarium to minimize glare and reflections (midday lighting in bright sunlight, say between 10 AM and 2 PM, works best but we could not always control the timing of our photo-shoots, nor the quality of the light). The aquarium itself was shifted and reoriented when necessary to eliminate shadows. We always placed a layer of clean gravel in the bottom of the aquarium (after first filling the tank with water to prevent scratching the Plexiglas) to avoid having the bottom of the unit show in our photographs, and we always shot against a neutral background which consisted of a light-blue backdrop cloth stretched over a board. Figure 3 shows the assembled unit in operation at streamside.

FIGURE 3

We shot two sets of photographs, a primary set and a backup set, of each fish. For the primary set, we Initially used an Olympus OM-1 manually operated camera equipped with a 28-to-80 mm macro-zoom lens. Proper exposure with this camera was problematic because the built-in light meter, although center-weighted, took into account all light entering the field of view and would underexpose the fish in bright light. To compensate for this, we took frequent exposure readings off a standard photographic grey card which we held against the wall of the aquarium, and then bracketed these readings by ± 1.5 ev (exposure value) units in 0.5 ev increments. Later, we replaced this camera with a Minolta Maxxum 600si camera equipped with autofocus and autoexposure features. With this camera, we could spot-meter the exposures directly off the side of the fish itself, which gave much more consistent results. Even so, we still used the camera's bracketing program to bracket the metered readings by ± 0.5 ev unit. All images in the primary photo set were made on 35-mm Kodachrome 200 transparency film, and all film was processed by the Kodak laboratory in Tukwila, Washington.

The backup photo set was taken to mimimize potential unforseen loss of film, accidents in processing, or equipment malfunction. A Nikon FE camera equipped with a 55 mm F 3.5 Micro-Nikkor lens was used with several film types that included Ectachrome Elite II at 100 ASA, Fujichrome Sensia at 100 ASA, Seattle Filmworks at 100 ASA, and Seattle Filmworks at 400 ASA. It proved necessary to use these backup photos as a choice for the appendix in a few instances.

Because of the tradition for taxonomic measurements to be made on the left side of the fish (Behnke 1992), we photographed all fish facing left.

A photo catalog of our collections was prepared by first selecting the best images (one each) of each fish. These images were sent to the Kodak laboratory for scanning onto a master compact disk. From the compact disk, the images were displayed individually on a computer and edited using Adobe Photoshop 4.0 software, and then converted to page format using Adobe Pagemaker 4.0.

Fin-Tissue Collection and Preservation

Our goal was to collect fin tissues from 20 specimens from each stream. We attained or exceeded this goal most of the time, but failed to do so at two sites late in the season during extreme low-water conditions. In the field, as noted above, we removed the adipose fin (or occasionally a caudal fin snippet of about the same size) from each fish collected. When collecting this tissue, we attempted at all times to retain the fin-clip on the scissors and avoid touching it with our fingers. We found this easiest to do if one of us held the fish and gently arched the back, thus presenting the adipose fin, while another person clipped the fin at the base, always approaching with the scissors from behind the fin. The fin-clip, now on the scissor tips, was then quickly transferred into a 2-mL vial filled with denatured 95-percent ethanol (Shiozawa et al. 1992). We used capped cryo-storage vials for this purpose, which had been pre-filled with ethanol and labeled with the site code and specimen number.

Shiozawa et al. (1992) have shown that adipose or fin tissue preserved in the field with 95-percent ethanol is particularly well suited to genetic analysis using DNA techniques. DNA analysis offers

several advantages when working with fish populations that are rare, threatened, or endangered and sacrificing fish must be avoided. Only very small quantities of tissue are needed, and these can be taken nonlethally. Also, DNA molecules are more stable than proteins under field and storage conditions. Tissue for DNA analysis need only be fixed in ethanol in the field—no dry ice or special logistics required—and the fixed tissue can be stored in a common refrigerator if need be until analyzed. Ethanol-fixed tissues are suitable for DNA extraction for several days when left at room temperature (Bramwell and Burns 1988) and for more than six years when kept at 4^0 C (Smith et al. 1987). In the present work, we kept the tissue vials in a Coleman cooler while still in the field, and stored them in a home freezer at -20^o C until they could be transported to the genetic laboratory for analysis.

Collection Site Physical Description and Habitat Data

After completing our protocols for fish photography, measuring, weighing, and tissue collection, we also recorded some basic measurements and observations of stream, riparian, and upland habitat condition at each collection site. Photographs were taken of the site at the outset, and subsequent photos were taken to pictorially record significant habitat features of each stream. One of these photos was chosen for each collection site to accompany the fish photos displayed in the appendix. We measured water temperature with hand-held thermometers at several times over the course of the day, and recorded the range. Gradient was measured with a Peco hand-held Abney level on one or more stream sections chosen as being typical of the overall collection reach. We also calculated stream discharge on the day of collection from measurements of water velocity and wetted channel width and depth. We also measured bankful width at many of the sites on the same transect used to compute discharge, using criteria set forth in Leopold (1994, p 131-133) and Leopold et al. (1995).

The procedure for obtaining stream discharge was to first measure the wetted channel width, then divide that transect into three to seven cells depending on the width. Water depth and water velocity were then measured at the mid-point of each cell. Water velocity readings were taken with a Global Systems “Flow Probe” hand-held flowmeter with the propellar immersed as best we could position it at 0.6 x water depth from the surface. The area of each cell was then determined by multiplying cell width by mid-point depth, and this was multiplied by that cell’s water velocity to get the discharge

for each cell. The sum of discharges for all cells across the wetted transect was taken as the total discharge for the channel at the time of measurement.

In order to further describe the sites as we found them, qualitative rankings of fourteen additional habitat parameters relating to riparian vegetation, streambank condition, bottom substrate, and channel condition were made by visual estimation using a three-page Habitat Assessment Field Data Form, originally developed for research on aquatic oligochaetes and other aquatic invertebrates by Dr. D. Kathman, Aquatic Resources Center, Franklin, Tennessee. Each habitat parameter (for both left and right banks where appropriate) was evaluated from a choice of four comparative values, and each of the four values was given a numerical score within a five point range: Poor (1-5), Marginal (6-10), Suboptimal (11-15), and Optimal (16-20). The maximum possible total score for a site was 360 points. Although subjective, this system was rapid and easy to use in the field, and provided a numerical means to compare individual habitat parameters among collection sites, and also to compare cumulative scores for each collection site.

A copy of the Habitat Assessment Field Data Form, showing the fourteen habitat parameters evaluated, is included in Appendix A. For consistency, the same person completed the ranking for all sites.

Genetic Analysis

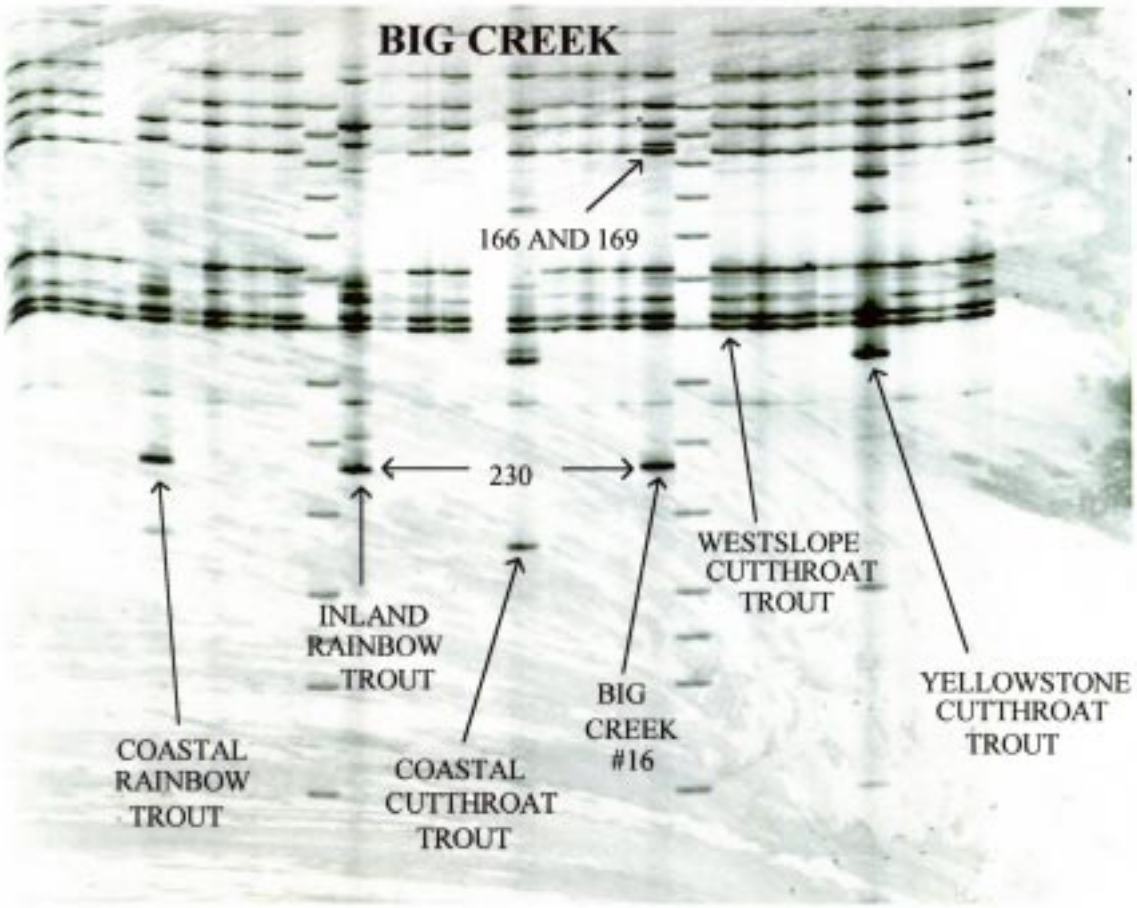
We used paired interspersed nuclear elements (PINEs) to identify species and subspecies of the fish collected, and to assess the extent of hybridization that might have occurred in the populations (Spruell et al. 1999). PINEs use pairs of primers that are complementary to the sequences of elements that are interspersed throughout the nuclear genome. Using the polymerase chain reaction (PCR), the fragments of DNA between these elements are amplified. When these amplified fragments are run on an electrophoretic gel, it is possible to reliably distinguish species based on the presence or absence of diagnostic bands. We used markers amplified by the same primer pairs to differentiate between coastal, Yellowstone, and westslope cutthroat trout and between coastal and inland rainbow trout.

With regard to hybridization between rainbow and cutthroat trout, PINEs do not always allow a distinction between inland and coastal forms of the rainbow trout component. This is because there are shared bands between the forms, and which of these bands will be expressed in the hybrid is random. Therefore, when levels of hybridization with rainbow trout are low, we cannot assign the hybrid influence to either form with certainty.

Fin clips stored in 95% ethanol, as described above, were transported to the University of Montana where DNA was extracted using guidelines provided with the Puregene™ DNA Isolation Kit. DNA was amplified using primers labeled with fluorescent dyes to allow visualization of the product. Each population was analyzed using a minimum of three primer pairs. PCR reagent volume was maintained at 10 µL. Reactions contained the following: approximately 25 ng of genomic DNA, 1 µL 10X Perkin-Elmer PCR buffer, 4.5 mM $MgCl_2$, 0.2 mM of each dNTP, 5.0 pmoles of primer, and 0.5 U Stoffel Taq. Reactions were completed in a MJ Research PTC-100 thermal cycler. All reactions except those including the primer 33.6+2 used the following profile: 3 minutes at 95°C, 30 cycles of: 1 minute at 93°C, 1 minute at 60°C, 2.5 minutes at 72°C, and finally an additional 2.5 minutes at 72°C. For reactions that included the primer 33.6+2, the 60° annealing temperature was increased to 61°. Products were then refrigerated until analysis on an electrophoretic gel. Amplified products were run on a 4.5% polyacrylamide gel for 50-75 minutes at 65 watts. DNA products were then visualized using a Hitachi FMBIO-100™ fluorescent imager.

We visually inspected each gel to identify DNA fragments that were diagnostic for interior or coastal rainbow trout, or for westslope, Yellowstone, or coastal cutthroat trout. The size of these bands was confirmed using MapMarker LOW size standard and FMBIO software. All gels also included at least one known individual from each species and subspecies in question to be used as a reference for the unknown samples. An example of a PINE gel is shown in Figure 4.

Figure 4


BIG CREEK
166 AND 169
230
WESTSLOPE CUTTHROAT TROUT
INLAND RAINBOW TROUT
BIG CREEK #16
YELLOWSTONE CUTTHROAT TROUT
COASTAL RAINBOW TROUT
COASTAL CUTTHROAT TROUT

RESULTS

Fish Abundance, Condition, and Other Factors Pertaining to Fish Collection

The results of our catch per unit of effort (cpue) and population mean Relative Weights for each collection site are presented in Table 2, along with collection date, stream discharge at the site on the day of collection, and a list of other salmonids encountered.

Table 2. CPUE, relative weights, and other salmonid fishes encountered, along with date of collection and stream discharge, Yakima Basin trout collections

Collection Site	Date	CPUE, no. per hr	W_r	Discharge m^3/sec	Other salmonids encountered
A N.F. Taneum Cr	6/22/98	18.4	84.14	0.736	Brook trout
B Cabin Cr.	6/29/98	9.4	79.14	0.397	
C Naneum Cr.	7/7/98	8.9	92.15	1.639	
D Wilson Cr.	7/9/98	5.8	82.34	0.538	
E Big Cr.	7/12/98	8.4	88.54	0.626	
F S.F. Toppenish Cr.	7/15/98	7.1	86.66	0.168	
G N.F. Ahtanum Cr.	7/23/98	6.7	92.52	0.166	Bull trout
H Cooke Cr.	7/29/98	6.9	80.66	0.172	
I M.F. Little Naches	8/21/98	5.0	82.45	0.374	Coho salmon
J Red Rock Cr.	9/9/98	1.1	86.52	0.173	Brook trout
K Meadow Cr.	9/22/98	3.1	81.36	NA	

We felt that cpue worked reasonably well as a surrogate for fish abundance until later in the season when flows became very low and our catch per unit of effort tailed off as well. Recreational anglers

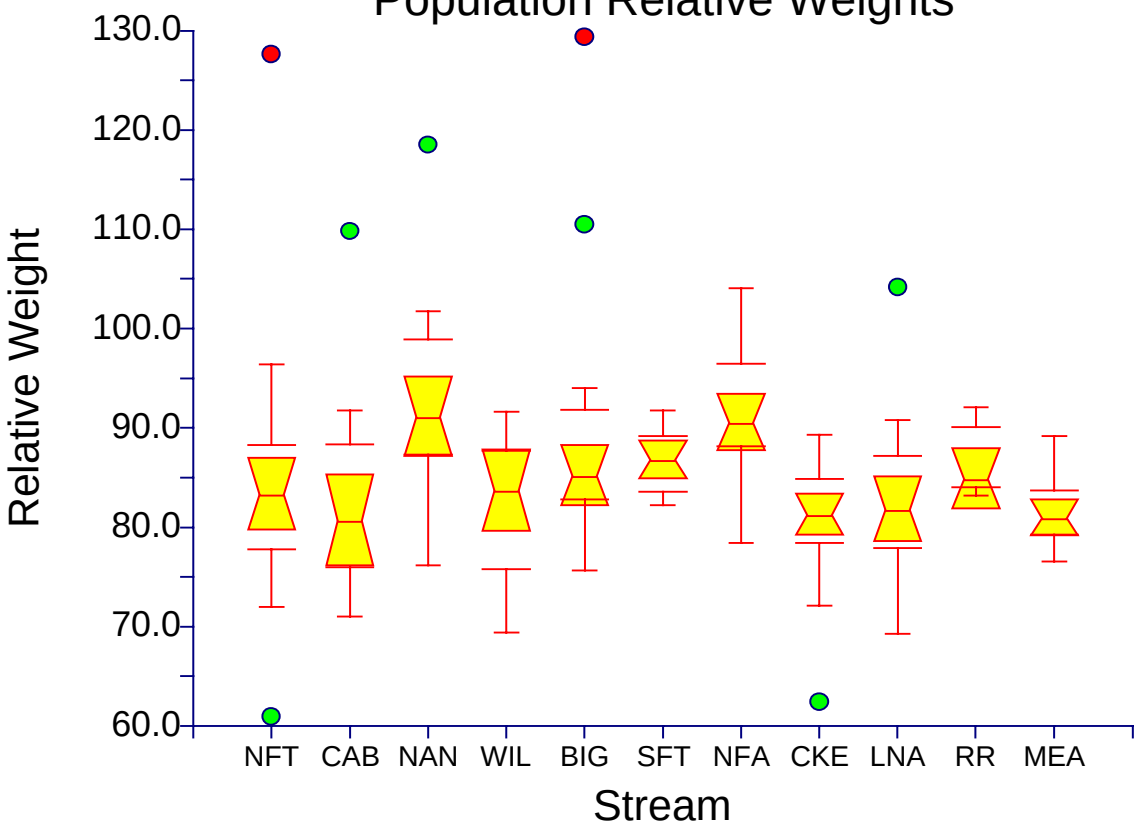
employing our methods would regard these cpue values as quite good for the most part, indicative of a high level of fish abundance. The one exception was Red Rock Creek. Even though we sampled there late in the season, our impression was that fish are not abundant in that stream.

As noted in Table 2, brook trout *Salvelinus fontinalis* were present along with cutthroat trout at our collection sites in North Fork Taneum and Red Rock creeks, although not abundantly so in either case. There is no record of brook trout stocking in North Fork Taneum Creek, but brook trout were released in the mainstem, many miles downstream of our collection site, in 1935 and 1937. Evidently, descendants of these fish have worked their way upstream over the years. No stocking records of any kind were found for Red Rock Creek, and none were found for brook trout in the small lake in its headwaters, but brook trout have been released in other parts of the upper Bumping River drainage. Migration into new territories by fish descended from those releases may account for the brook trout we caught at the Red Rock Creek site. Bull trout *Salvelinus confluentus* were encountered in sympatry with cutthroat trout at the North Fork Ahtanum Creek site. This is a known population (WDFW 1998), so the few that we encountered were not unexpected.

The presence of coho salmon in the upper Little Naches drainage did come as a surprise. While reconnoitering for a collection site on several streams in this drainage reported to be inhabited by cutthroat trout (Hubble et al. 1990), we instead encountered masses of coho parr, but no trout whatsoever—except a single juvenile cutthroat trout found in the South Fork Little Naches River and in one reach of the Middle Fork Little Naches where coho parr were absent and cutthroat trout were finally located in numbers sufficient to complete our collection. Later we learned that these coho parr were out-of-basin hatchery fish, planted in an effort to reintroduce coho to the Yakima River basin where they have been extinct for many years (David Lind, Yakama Indian Nation, personal communication 1998). However, the outplanting of coho parr seems to have been quite heavy, with the apparent effect of displacement or emigration of resident trout from the stocked reaches. We will revisit this observation later in the Discussion section.

Population mean Relative Weights for the fish collected are shown in notched box-plot form in Figure 5.

Figure 5
Population Relative Weights



Notched Box Plots of Relative Weights of the sampled stream populations.
The top and bottom of the "box" are the 25th and 75th percentiles and are indicated by horizontal lines nearest the box but attached to the T-shaped lines which extend away from the box. The length between these two lines on either side of the box itself is thus the interquartile range (IQR, the middle 50% of the data).
The line drawn through the middle of the box is the median (the 50th percentile). The outer limits of the notched box itself display the 95% confidence limits of the median, and are constructed using the formula: *Median* ± 1.57*[(IQR)/√n].
Adjacent values are displayed as T-shaped lines that extend beyond each end of the box. The upper adjacent value is the largest observation that is less than or equal to the 75th percentile plus 1.5 times IQR. The lower adjacent value is the smallest observation that is greater than or equal to the 25th percentile minus 1.5 times IQR. Values outside the upper and lower adjacent values are called outside values; those that are under three IQRs from the 25^{th} and 75^{th} percentiles are called "mild outliers" (green dots), those outside three IQRs are called "severe outliers" (red dots).

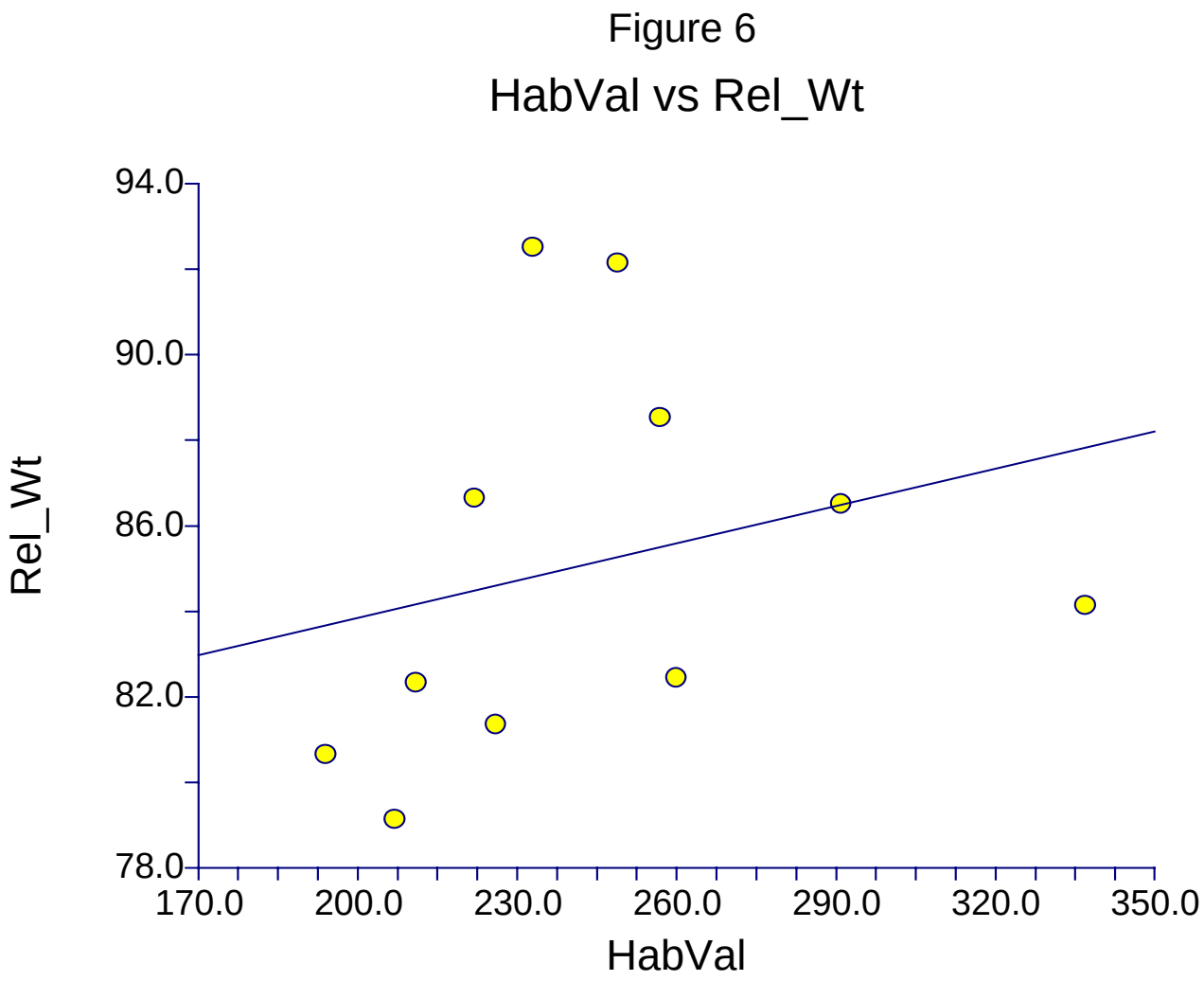
Notched boxes span the 95% confidence interval around the sample median which is depicted as the midline of the box. Boxes which don't overlap can be visually determined to have different median values at the $\alpha = 0.05$ significance level.

Results of statistical tests reject the null hypothesis that all population means are equal at the significance level $\alpha = 0.05$ (One-Way Anova; df (degrees of freedom) = <10,205>, F-Ratio = 5.36, p = 0.000000) and the null hypothesis that population medians are equal (Kruskal-Wallis One-Way Anova on Ranks; df = 10, Chi-Square = 59.02156, p = 0.000000).

There was no evident correlation between population mean Relative Weight and bi-weekly period of sampling (Nested Anova, df: Sampling Periods = 5, Streams = 5, Error Term = 205; F-Ratio, Sampling Periods/Streams = 0.50, p = 0.77; F-Ratio, Streams/Error = 7.17, p = 0.000003).

Nor did there appear to be any correlation between Relative Weights and location within the basin, elevation, or habitat quality score. However, the scatter plot of population mean Relative Weights against habitat quality scores, shown in Figure 6, does suggest that the highest Relative Weights occurred in streams with intermediate scores between 230 and 260 in the rating system we used.

Figure 6



Results of descriptive statistics and Anovas run on the length data of the samples support our conviction that our method of sampling produced random samples of the populations. Of the 11 populations sampled, 10 had normal distributions according to the D'Agostino Omnibus normality test at the $\alpha = 0.05$ significance level. Means of the 10 normal samples ranged from 136 to 189 mm fork-length, sample standard deviations ranged from 17.4 to 34.5, and coefficients of variation ranged from 0.124 to 0.213. The North Fork Taneum Creek sample contained two outliers at the right end of the length distribution which resulted in the sample failing the normality test. The mean of this population was 152.6mm, the sample standard deviation was 39.7, and the coefficient of variation 0.26. Results of the descriptive statistics are summarized in Table 3 .

Table 3. Stream Sample Fork-Length Summary Statistics

Stream	Mean(mm)	Std. Dev.	C.V.	Normal?
NFT	152.6	39.7	0.260	N
CAB	186.4	32.6	0.175	Y
NAN	169	32.2	0.191	Y
WIL	183.2	34.5	0.188	Y
BIG	156.4	24.8	0.159	Y
SFT	138.9	17.4	0.125	Y
NFA	171.3	27.5	0.161	Y
CKE	147.6	31.5	0.213	Y
LNA	136.2	24.3	0.178	Y
RR	173.9	21.6	0.124	Y
MEA	189.1	34.3	0.181	Y

Anovas on the fork-length data paralleled the Anovas on the Relative Weight data. The null hypothesis that all sample means were the same was rejected at the significance level $\alpha = 0.05$ (One-Way Anova, df=<10,205>, F-Ratio = 7.60, p = 0.00000). In addition, the Modified-Levene Equal-Variance Test accepted the null hypothesis of equal variance among the samples at the $\alpha = 0.05$ (p = 0.363). The Nested Anova found no difference among population means grouped by bi-weekly period of sampling (dfs: Sampling Periods = 5, Streams = 5, Error Term = 205; F-Ratio, Sampling Periods/Streams = 0.70, p = 0.65; F-Ratio, Streams/Error = 8.94, p = 0.000000).

Results of correlation and least squares regression of sample Relative Weights on fork-lengths were not significant for eight (8) of the eleven (11) samples at the $\alpha = 0.05$ level. Regression line slopes for theses eight samples ranged from –0.11 to 0.181 and R^2 values ranged from 0 to 0.11, which were not significantly different from zero. Modest negative correlations were found for two samples. Cabin Creek had identical Pearson and Spearman-Rank correlation values of –0.49 ($p = 0.03$) and a regression line slope of –0.21, ($R^2 = 0.24$). Wilson Creek had a Pearson correlation value of –0.57 ($p = 0.015$), a Spearman-Rank correlation value of –0.52 ($p = 0.008$) and a regression line slope of -0.10 ($R^2 = 0.27$). The final sample (Middle Fork Little Naches river) had a positive Pearson correlation value of 0.43, which had a barely insignificant p-value of 0.06. The Spearman-Rank correlation values was 0.32, which was insignificant ($p = 0.17$). The slope of the regression line was 0.15, with an R^2 value of 0.18.

Photo Catalog of Trout Specimens

The photo catalog of live specimens representing each of the collected trout populations, along with a photograph of each respective collection site, is included in Appendix B.

Results of DNA Analysis

The results of the DNA analysis are presented in Table 4. Five of the ten cutthroat trout populations collected were found to be pure *lewisi*. These are: Site B, Cabin Creek; Site C, Naneum Creek; Site F, South Fork Toppenish Creek; Site G, North Fork Ahtanum Creek; and Site K, Meadow Creek. The remaining collections exhibited low to moderate levels of hybridization with rainbow trout based on number of hybrid individuals in the population—from 4.5 percent (Site E, Big Creek) to 20 percent (Site J, Red Rock Creek). However, all were judged to be "good" representatives of westslope cutthroat trout appearance-wise, given the variability we found in spotting patterns (reported below). The distribution of these pure and hybridized cutthroat trout populations within the Yakima Basin is mapped in Figure 7.

Figure 7
Distribution of Pure and Hybridized Cutthroat Populations within the Yakima Basin

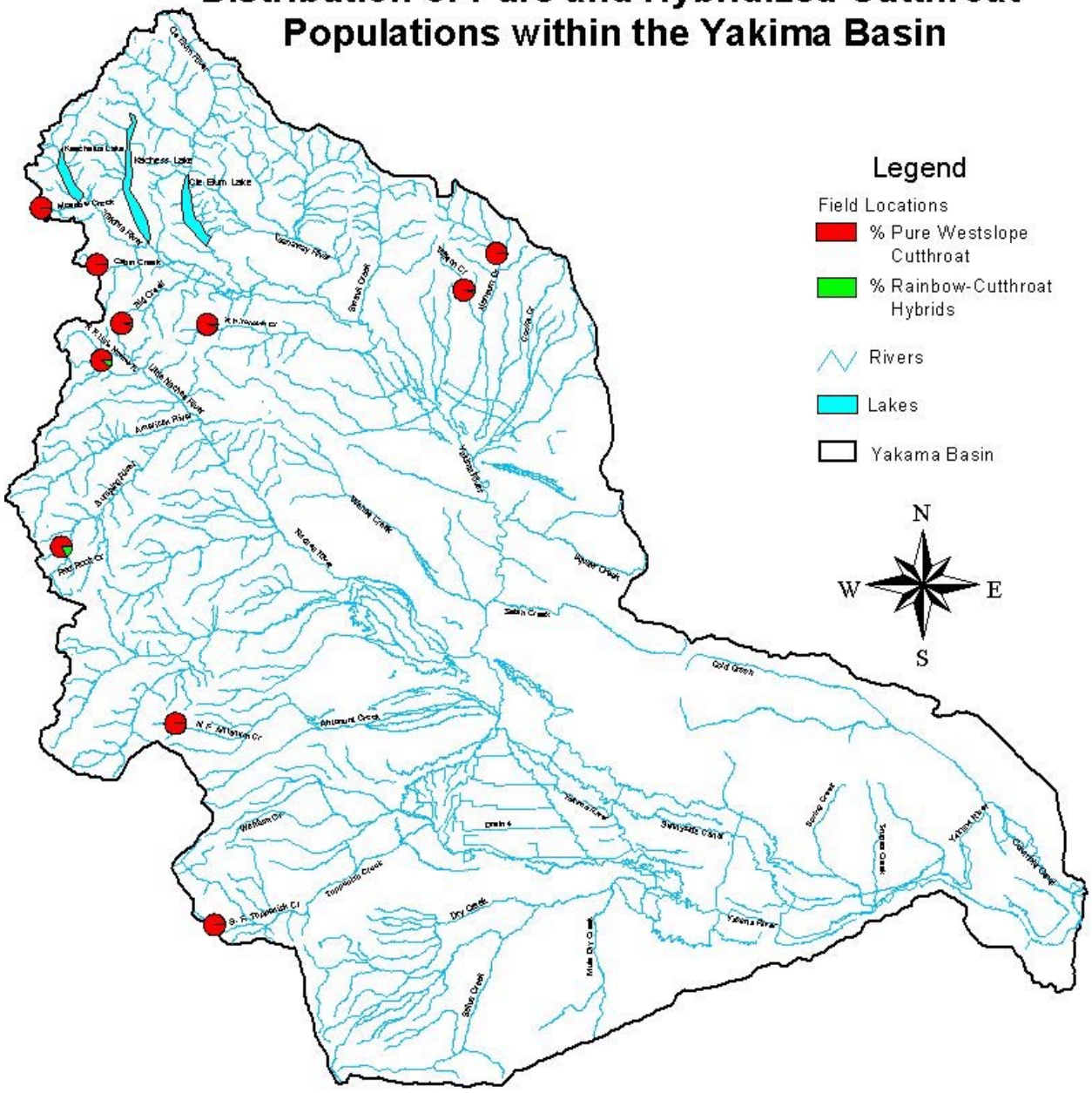


Table 4. Genetically pure and hybridized populations, Yakima Basin trout collections

Collection Site	Number of Specimens	Number of Hybrids	Percent Rainbow Bands In Hybrids
A N.F. Taneum Cr.	21	1	28.57%
B Cabin Cr.	20	0	0
C Naneum Cr.	21	0	0
D Wilson Cr.	21	1	14.29%
E Big Cr.	22	1	42.86%
F S.F. Toppenish	21	0	0
G N.F.Ahtanum Cr.	21	0	0
I M.F. Little Naches	20	2	42.86%, 57.14%
J Red Rock Cr.	10	2	14.29%, 28.57%
K Meadow Cr.	15	0	0
H Cooke Cr.	24	0	(See text)

Cooke Creek, Site H, was reported to us to be an interior rainbow trout site. Our results indicate that this is not entirely the case. No hybrid individuals were found among the specimens collected from Cooke Creek, but rather, a mixture of rainbow trout subspecies was found. Seventy-five percent of the individuals collected were pure interior rainbow trout (subspecies *gairdneri*) and 25 percent of the individuals collected were pure coastal rainbow trout (subspecies *irideus*). This result indicates the presence of hatchery origin fish in the population, probably the descendants of fish stocked during the period from 1936 to 1973. The record shows that every year during that period except 1972, from 500 to 2000 hatchery rainbow trout were released into Cooke Creek, with no fish of any kind stocked after 1973. The presence of *irideus* in the stream 25 years later indicates that some of

those hatchery fish must have reproduced. However, as none of the fish we collected were hybrids, interbreeding has evidently not yet occurred in upper Cooke Creek.

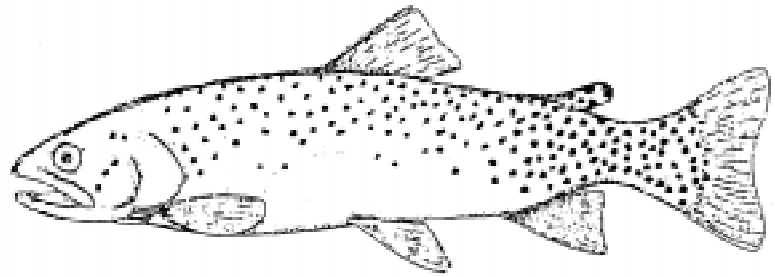
Westslope Cutthroat Spotting Pattern Phenotypes

The spots of westslope cutthroat trout are most often described as small and irregular in outline, with few spots occurring on the anterior body below the lateral line (Behnke 1992). The area within an arc extending from the origin of the pectoral fin to just above the lateral line then downward to the origin of the anal fin usually has very few or no spots in westslope cutthroat trout (Behnke 1992). This distribution of body spots, which we labeled the "classic fine-spotted" pattern and illustrate in Figure 8A, is said to be consistent throughout the subspecies range (Behnke 1992).

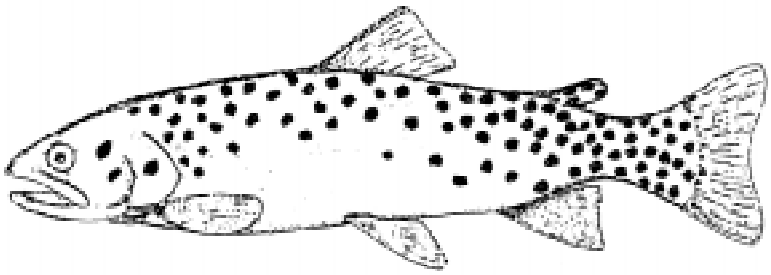
However, in this study we observed at least four additional spotting patterns, even on cutthroat trout found to be pure *lewisi* by our DNA analysis. We labeled these the "classic large-spotted" pattern (Figure 8B); the "minimal fine-spotted" pattern (Figure 8C); the "minimal large-spotted" pattern (Figure 8D); and the "leopard-spot" pattern (Figure 8E). Occassionally, one of these other patterns rather than the "classic fine-spotted" pattern would be the predominate pattern among the fish in the collection. This was true in the Big Creek collection, for example, where the "minimal large-spotted" pattern predominated. In other cases, we observed several spotting patterns occurring together in the stream, with none of the patterns predominating. Photographs of fish bearing each of these patterns can be found in the photo catalog in Appendix B.

The spotting patterns we have labeled "leopard-spot" (Figure 8E) and "minimal large-spotted" (Figure 8D) are of particular interest, because these are patterns one would expect to see on another interior cutthroat trout subspecies, namely the Yellowstone cutthroat *Oncorhynchus clarki bouvieri* (Behnke 1992; Jordan and Evermann 1902). Records show that many shipments of *bouvieri* eggs were indeed made from Yellowstone Lake to both Kittitas and Yakima Counties in the years between 1916 and 1950 (Varley 1979), but we could find no record of where these fish were stocked. Although this record of shipments to the basin exists, no evidence of introgression by *bouvieri* was found in our DNA analysis.

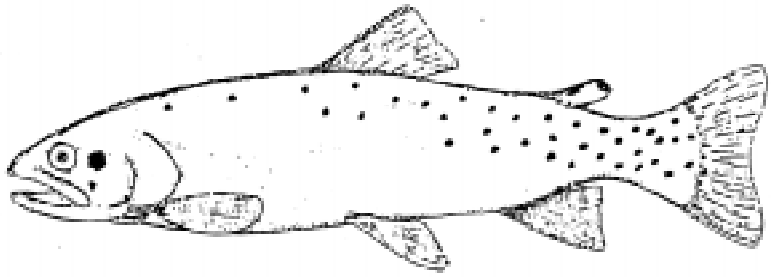
Figure 8. Spotting phenotypes observed in Yakima basin westslope cutthroat trout (dorsal and caudal spotting not shown).



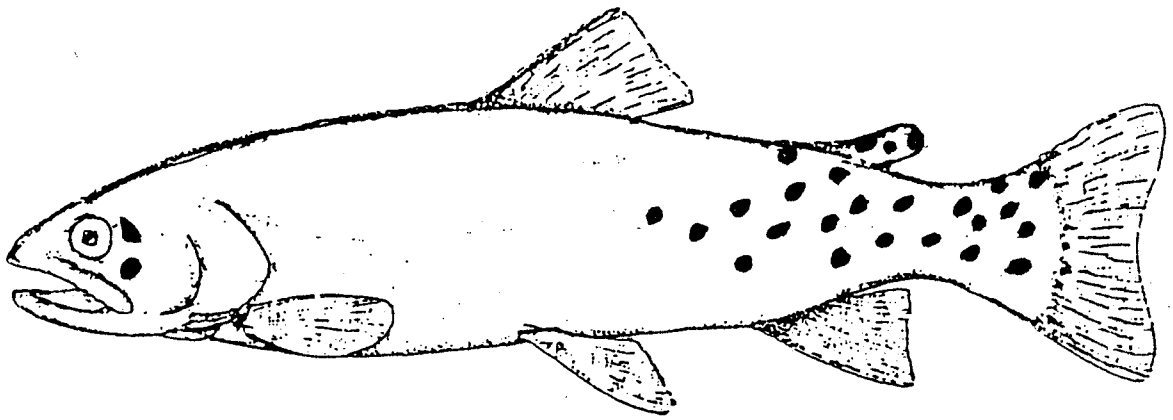
A. Classic fine-spotted



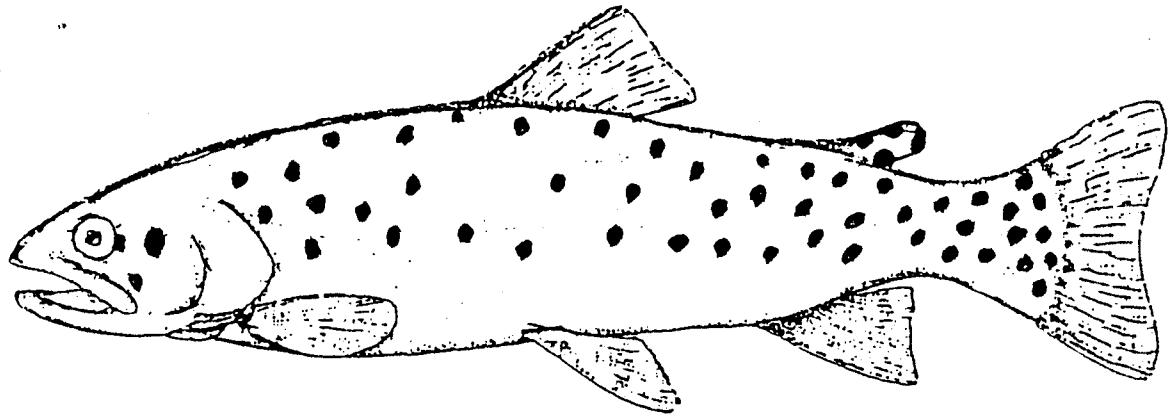
B. Classic large-spotted



C. Minimal fine-spotted



D. Minimal large-spotted



E. Leopard-spot

DISCUSSION

Half of the cutthroat trout populations examined in this study—five out of ten populations—proved to be genetically pure westslope cutthroat trout. In two of these cases, Cabin Creek and South Fork Toppenish Creek, no record of cutthroat trout stocking in these or any nearby waters could be found, a fact we take to be evidence that the fish we examined are native to those streams. This finding lends support to earlier suggestions (Behnke 1992; Proebstel and Noble 1994) that the range of the westslope cutthroat trout extends into central Washington State westward to the Cascade crest. It

also suggests that the evolutionarily younger and later-invading rainbow trout (Behnke 1992) has not completely displaced native cutthroat from the Yakima basin, especially not from the uppermost reaches of trout-bearing waters of the the North Cascades, Cascades, and Eastern Cascades Slopes and Foothills ecoregions. On the other hand, it is true that rainbow trout do occupy the lower reaches of these same streams as well as the mainstem Yakima itself (Campton and Johnston 1985; Phelps 1993; Proebstel 1998). Partitioning of stream reaches in this manner—cutthroat trout in the headwater reaches and rainbow trout in the lower reaches—is common where resident forms of the two species co-occur naturally (Hartman and Gill 1968; Hanson 1977; Robert H. Smith, personal observation cited in Behnke 1992, page 80). It is also true that Cooke Creek, the easternmost stream from which we collected, has only rainbow trout as do all other streams east of Cooke Creek (G. McMichael, Washington Department of Fish and Wildlife, Ellensburg, Washington, personal communication 1998). Cooke Creek is located at the boundary between the Eastern Cascades Slopes and Foothills ecoregion and the more xeric Columbia Plateau ecoregion (Pater et al. 1998), which may offer at least a partial explanation for the transition from cutthroat trout to rainbow trout as the principal occupants of headwater reaches in the respective streams.

Our results further suggest that the Toppenish Creek drainage may be the present southern limit of the westslope cutthroat range in Washington. Only brook trout have been found in the few surveys made to date in upper tributaries of the Satus Creek drainage, which is the next drainage south of Toppenish Creek (D. Lind, Yakama Indian Nation Fisheries, personal communication 1998). Westslope cutthroat trout are, of course, known to occur even further south, in tributaries of the John Day River in Oregon (Behnke 1992).

As for the three remaining genetically pure westslope cutthroat populations, we found stocking records stating that cutthroat trout—origin and subspecies not specified in the record—were planted somewhere in those systems. In each of these cases, the stocking record mentions only a single release, or at most two releases of hatchery cutthroat trout, and each of those releases was made decades ago. Even so, the argument can and has been made (see, for example, Crawford 1998) that the cutthroat trout present there now resulted from those stocked fish. One cannot refute such an argument, but frankly, we believe it stretches the available evidence. It is just as likely in our view that those old plantings could have been made in streams already inhabited by native trout. It was

not always true that early-day fishery managers sought out only streams barren of fish to stock with hatchery trout. A philosophy also prevailed among fish culturists and managers of those times that the best places to plant hatchery trout were in streams where trout were already present. After all, what better demonstration could there be of the ability of a stream to support trout? One can find this philosophy stated often in old reports of the Washington State Fish Commissioner (Washington State Fish Commissioner 1905–1919).

We also found five westslope cutthroat populations that exhibited low levels of hybridization with rainbow trout. In three of these populations, less than 5 percent of the individuals were hybrids. In two other populations the percents of hybrid individuals were 10 percent and 20 percent respectively. As it happened, four of the hybrid individuals were among the fish photographed in the portable aquarium and included in the photo catalog in Appendix B. These are specimens AA-19 (North Fork Taneum Creek), DA-2 (Wilson Creek), JA-2 and JA-5 (both from Red Rock Creek). Looking at these photos in retrospect, one can, perhaps, find subtle hints of hybrid influence. However, in the field, before the results of the genetic tests were known, we had confidently identified each of these trout as a westslope cutthroat based on its visual appearance, in part due to the great variability found in the spotting patterns of cutthroat in the Yakima drainage which made identification of hybrid characteristics more difficult.

To help his colleagues cope with this same kind of situation in the management of another interior cutthroat subspecies, the Colorado River cutthroat trout *O. clarki. pleuriticus*, Dr. Niles Allen Binns of the Wyoming Game and Fish Department devised a purity rating system using a scale of A (pure) to F (obvious hybrid) (Binns 1977). In southwestern Wyoming, where this system was first applied, only a few pure populations of *pleuriticus* were known to exist but many hybridized populations could be found that were, despite hybridization, visually "good" representatives of the subspecies. Some sort of purity rating system was needed to help justify measures to protect the few remaining pure stocks, and to guide the selection of populations to use in restoration efforts. The Binns system was devised when comparison of meristic character measurements was the principal method of detecting hybridization. Although today's methods are more sophisticated, still, it seems appropriate to consider the Binns approach for the Yakima River basin, where cutthroat trout brought to hand

appear to be “good” representatives of the westslope cutthroat subspecies but varying amounts of hybridization with rainbow trout has occurred in some populations.

The Binns system as originally promulgated was as follows:

A. Pure stock. No trace of hybridization in meristic characters and no history of stocking the water with non-native species.

B. Only a trace of hybrid influence detectable in meristic characters, but appearance-wise a “good” representative of the species or subspecies of interest. Also applied to populations with no detectable hybridization from meristic character comparison, but a history existed of stocking the water with non-native species.

C. Hybrid influence obvious from meristic characters, but still appearance-wise a “good” representative of the species or subspecies.

D. Definite hybrid evident from meristic characters but still visually a “good” species or subspecies representative.

E. A population not examined by a taxonomist.

F. Obvious hybrid both in meristic characters and in appearance. A poor visual representative of the species or subspecies of interest.

We modified this basic framework in the following ways to make it applicable to our DNA analysis:

A. Pure stock. All individuals examined carry only markers of the species or subspecies of interest, and there is no history of stocking the water with hatchery fish of the same species or subspecies.

B. 1-9 percent of individuals examined carry bands from another species or subspecies, but all are "good" representatives of the species or subspecies appearance-wise. Also applied to populations with no detectable hybridization, but a history exists of stocking the water with hatchery fish of the same species or subspecies.

C. 10-19 percent of the individuals examined carry bands from another species or subspecies, but all are still "good" visual representatives of the species or subspecies of interest.

D. 20 percent or more of the individuals examined carry bands from another species or subspecies, but all are still "good" visual representatives of the species or subspecies of interest.

E. A population never examined by a taxonomist or by any genetic method.

F. 20 percent or more of the individuals examined carry bands from another species or subspecies, and the specimens are questionable to poor visual representatives of the species or subspecies of interest. This designation would also apply to populations that are hybrid swarms.

Because we can easily detect foreign DNA bands in the specimens we examine, we dropped that part of Binns' stocking history criterion that downgraded populations from A to B if there was a record of stocking with a different species or subspecies, e.g., rainbow trout stocked in a cutthroat trout stream, or Yellowstone cutthroat stocked in a westslope cutthroat stream. Instead, we chose to downgrade due to stocking history alone only if the following conditions exist: (1) if the record showed that non-indigenous fish of the same species or subspecies were stocked, e.g., Twin Lakes strain westslope cutthroat stocked in a stream where we found pure westslope cutthroat trout; or (2) if the stocked fish were the same species but were not identified as to subspecies or origin, e.g., fish identified only as "cutthroat trout" stocked in a stream where we found pure westslope cutthroat. Table 4 shows the outcome of applying our modified Binns classification to the FY-1998 cutthroat trout collections.

Table 5. Hybridization and purity ratings for Yakima Basin cutthroat trout collections

Collection Site	Percent Hybrids with Rainbow	Record of Stocking with Cutthroat	Modified Binns Class
A N.F. Taneum Cr.	4.8	1936, 1937	B
B Cabin Cr.	0	No	A
C Naneum Cr.	0	1914, 1933	B
D Wilson Cr.	4.8	1937	B
E Big Cr.	4.5	1914	B
F S.F. Toppenish	0	No	A
G N.F.Ahtanum Cr.	0	1967, 1971	B
I M.F. Little Naches	10	No	C
J Red Rock Cr.	20	Two Lake 1962–1989	D
K Meadow Cr.	0	1938	B

Thus, although half of the westslope cutthroat populations (five populations) examined in this study are pure *lewisi*, only two of these populations, Cabin Creek and South Fork Toppenish Creek, meet both criteria for the A-rating (no hybrids and no record of cutthroat stocking). The other three pure *lewisi* populations (Naneum Creek, North Fork Ahtanum Creek, and Meadow Creek) were downgraded to B because hatchery cutthroat trout of unspecified origin and identity were stocked in these waters. Three additional populations (North Fork Taneum, Wilson, and Big creeks) were rated B because of low levels of hybridization with rainbow trout. The Middle Fork Little Naches River population was rated C because of a 10 percent level of hybridization with rainbow trout, and the Red Rock Creek population was given a D-rating because of a 20 percent level of hybridization with rainbow trout. We reiterate, however, that in each of these cases the individuals we inspected in the field appeared to be "good" representatives of the subspecies *lewisi* appearance-wise.

Ironically, the most-hybridized population among the FY-1998 collections was the cutthroat population from Red Rock Creek, a site that happens to be located in a designated Wilderness. This demonstrates that occurrence in a protected area alone does not guarantee genetic purity, especially if the Wilderness designation was imparted recently. A great deal of stocking of lakes and streams in the Red Rock Creek area occurred prior to Wilderness designation, and afterward as well. This stocking has, unfortunately, had an adverse influence on the genetic purity of this particular population.

With regard to hybridization between cutthroat and rainbow trout, PINEs do not allow a confident determination of which form of rainbow trout has contributed to the mixed breeding when the population-wise level of hybridization is low. In the hybrid specimen from Big Creek, illustrated in Figure 4, the rainbow trout contribution appears to be clearly from the native redband subspecies. However, because there are shared bands between the rainbow subspecies, and which of these bands will be expressed in the hybrid is random, we cannot, as a general rule, assign the hybrid influence to either form with certainty. In other areas where westslope cutthroat and Columbia River redband rainbow trout occur sympatrically, hybridization between them (as detected through the use of allozyme electrophoresis) appears to have rarely if ever occurred (Sage et al. 1992; Leary 1997). Hybrid swarms were encountered occassionally in these other areas, but in those cases the fish always contained genes from coastal rainbow trout as well as genes from the two native species. This led Leary (1997) to conclude that the introduced coastal rainbow trout disrupted the reproductive isolation that naturally existed between the two native species, resulting in the formation of hybrid swarms and the loss (from a genetic standpoint) of the native species. Our study differs from Sage et al. (1992) and Leary (1997) in that we found only low levels of hybridization, not hybrid swarms—and never any pure rainbow trout of either subspecies—among our cutthroat trout collections. Given the uncertainty of PINEs in sorting out low levels of rainbow hybridization, we can add no further insights into the impact on native species of hatchery rainbow stocking in the Yakima basin.

As we indicated earlier in this discussion, Cooke Creek, the easternmost stream from which we collected, has only rainbow trout in its upper reaches, as do all other streams east of Cooke Creek (G. McMichael, Washington Department of Fish and Wildlife, Ellensburg, Washington, personal

communication 1998). The location of Cooke Creek at what appears to be a range boundary, where westslope cutthroat trout have given way completely to the evolutionarily younger and later-invading Columbia River redband rainbow trout even in the uppermost reaches of fish-bearing water, offers a unique opportunity for further research into possible mechanisms of such displacements. The boundary between the Eastern Cascades Slopes and Foothills ecoregion and the more xeric Columbia Plateau ecoregion (Pater et al. 1998) is also mapped here, which could offer at least one line of research into causal mechanisms for species distributions.

One could also use the streams in this particular boundary area to study how indigenous species can resist or yield to replacement by an introduced species such as brook trout. The upper reaches of Coleman Creek, the first drainage west of Cooke Creek, are isolated from the lower reaches by an impassible waterfall some 20 m (approximately 60 ft) high. Although rainbow trout inhabit the lower reaches, the isolated upper section was reported to be cutthroat trout water (G. McMichael, Washington Department of Fish and Wildlife, Ellensburg, Washington, personal communication 1998). We explored the upper reaches of Coleman Creek in July, 1998 in search of the cutthroat trout, but found only brook trout there instead. Brook trout were stocked in Coleman Creek on five occasions, in 1933, 1934, 1935, 1941, and 1956, and were evidently successful enough in this setting to completely replace the indigenous cutthroat trout. On the other hand, Naneum Creek and Wilson Creek, the next two drainages west of Coleman, have similar brook trout stocking histories, but in those drainages brook trout have not replaced the indigenous cutthroat trout. It would be interesting, we believe, to more deeply analyze the characteristics of each of these drainages to try to understand what governs the relative success of the three salmonid species.

One other item that merits comment in this discussion is our observation that cutthroat trout were absent from previously reported cutthroat-bearing stream reaches of the Middle Fork Little Naches River following heavy outplanting of hatchery coho parr, but were congregated in a reach of the same stream where coho parr were absent. This suggests that cutthroat trout may have abandoned the heavily planted reaches. Others have observed similar emigrations, for example, of coastal cutthroat trout in the face of heavy outplantings of hatchery coho parr (Tripp and McCart 1983; Peters et al. 1996). Indeed, increased competition from hatchery coho parr may pose a prominent risk for juvenile coastal cutthroat trout (Trotter et al. 1993; Johnson et al. 1999). To illuminate this

further, the National Marine Fisheries Service (NMFS) did a graphical analysis of trends in coastal cutthroat trout abundance in Washington streams, comparing those where hatchery coho fry have been continually released with those without coho fry plants over the same period of time. NMFS found that a majority of streams with continuing coho plants show declining trends in coastal cutthroat trout abundance while streams with no coho plants had mixed trends in abundance, some declining, others steady, and some increasing over the period (Johnson et al. 1999, their Fig. 34). Our observation suggests that the outplanting of hatchery coho parr could pose a similar risk for westslope cutthroat trout populations. The management implication of this should be obvious, in the face of a potential listing of westslope cutthroat trout under the U. S. Endangered Species Act. Managers interested in the reintroduction of coho salmon to the Yakima River basin will need to select their outplanting sites with great care to avoid the inadvertent displacement of pure stocks of westslope cutthroat trout.

Finally, a few observations on the condition of some of the populations that were sampled. The Cabin Creek cutthroat population had the lowest population mean, a result explained by several indicators that the fish had spawned very recently (unusually high levels of stress in fish brought to hand; loose eggs in gravel patches), probably within just a few days of our collection trip. The high levels of stress in samples from this population were manifested in an unexpected and unprecedented number of mortalities (8 of 20) during post-work-up recovery, the only mortalities encountered during the entire season's sampling. This was likely also manifested in the statistically significant negative correlation between Relative Weight and fork-length noted previously; larger, sexually mature fish were in markedly poorer condition than smaller fish.

While Cabin Creek was one of the northern-most sample streams, located on the southeastern flanks of the Cascades, it was among the lowest elevation streams sampled and recorded the highest mid-day water temperature of any stream sampled during the period between June 22 and July 12 (Table 1). Thus, the presence of spawning fish was quite unexpected. North Fork Taneum Creek, sampled a week earlier, and Big Creek sampled nearly two weeks later were higher in elevation and colder in water temperature, but evidenced no sign of spawning having been completed during the week prior to our visit. Cabin Creek also received the lowest overall qualitative habitat score of the ten westslope populations sampled.

Noteworthy with regard to habitat quality is Cooke Creek. It was the only site where interior rainbow trout were collected, and, from Table 1, it placed last among the collection sites in habitat quality score. Trout were relatively abundant in Cooke Creek (cpue of 6.9 from Table 2), but the population mean Relative Weight of 80.66 was the second lowest recorded. Our field notes indicate that small and presumably younger-age fish dominated the population, with only a small number of larger and presumably older, spawning-age fish present. The notes also reveal that Cooke Creek scored high in riffle categories of the habitat assessment, but low in pool variability. It appeared to be a more heavily grazed site than we found elsewhere, and the area appeared to have been recently logged. The apparent dominance of younger-age fish may also be indicative that the population is in the process of recovery from a recent major disturbance event. The regression line slope of W_r on length of -0.024 (not statistically different from zero) reflected the fact that the entire length spectrum of sampled fish contributed more or less equally to the relatively low mean condition of this population. This is consistent with the hypothesis of limitation by general (local) habitat/environmental conditions. We suspect Cooke Creek has warmer average water temperatures in summer than most of the other creeks sampled. On the day we visited, we recorded a water temperature of 15.8 degrees C. on a 33 degrees C.-plus afternoon. Although this was one of the warmest days experienced at any of the collection sites, it was the highest water temperature we recorded.

REFERENCES

American Wildlands and 6 co-petitioners. 1997. Petition for a rule to list the westslope cutthroat trout (*Oncorhynchus clarki lewisi*) as threatened throughout its range. Bozeman, Montana, American Wildlands and 6 co-petitioners.

Anderson, W. G., R. S. McKinley and M. Colavecchia. 1997. The use of clove oil as an anesthetic for rainbow trout and its effects on swimming performance. North American Journal of Fisheries Management 17: 301-307.

Behnke, R. J. 1992. Native trout of western North America. Bethesda, Maryland, American Fisheries Society Monograph 6.

Binns, N. A. 1977. Present status of indigenous populations of cutthroat trout, *Salmo clarki*, in southwest Wyoming. Cheyenne, Wyoming Game and Fish Department, Fishery Technical Bulletin 2.

Bramwell, N. H. and B. F. Burns. 1988. The effects of fixative type and fixation time on the quantity and quality of extractable DNA for hybridization studies on lymphoid tissue. Experimental Hematology 16: 730-732.

Bryant, F. G. and Z. E. Parkhurst. 1950. Survey of the Columbia River and its tributaries, Part IV. U. S. Fish and Wildlife Service, Special Scientific Report 37.

BuRec 1998. Yakima River basin water enhancement project, Washington: draft programmatic environmental impact statement. U. S. Department of the Interior, Bureau of Reclamation, Pacific Northwest Region, Upper Columbia Area Office, Yakima, Washington.

Campton, D. E. and J. M. Johnston. 1985. Electrophoretic evidence for a genetic admixture of native and nonnative rainbow trout in the Yakima River, Washington. Transactions of the American Fisheries Society 114: 782-793.

Carlander, K. D. 1969. Handbook of freshwater fishery biology. Volume 1. Ames, Iowa, The Iowa State University Press.

Cone, R. S. 1989. The need to reconsider the use of condition indices in fishery science. Transactions of the American Fisheries Society 118: 510-514.

Crawford, B. A. 1979. The origin and history of the trout broodstocks of the Washington Department of Game. Olympia, Washington State Game Department, Fishery Research Report.

Crawford, B. A. 1998. Status review of westslope cutthroat trout in Washington. Olympia, Washington Department of Fish and Wildlife, cover letter and attached report to L. Kaeding, U. S. Fish and Wildlife Service.

Fuhrer, G. J. and 6 co-authors. 1996. Surface-water-quality assessment of the Yakima River Basin in Washington: spatial and temporal distribution of trace elements in water, sediment, and aquatic biota, 1987-1991. U. S. Geological Survey, Open-File Report 95-440.

Hanson, D. L. 1977. Habitat selection and spatial interaction in allopatric and sympatric populations of cutthroat and steelhead trout. Ph.D. dissertation, University of Idaho, Moscow.

Hartman, G. A. and C. A. Gill. 1968. Distribution of juvenile steelhead and cutthroat trout (*Salmo gairdneri* and*S. clarki clarki*) within streams in southwestern British Columbia. Journal of the Fisheries Research Board of Canada 25: 33-48.

Hintze, J. L. 1999. Users Guide–NCSS 2000, Number Cruncher Statistical System for Windows. Kaysville, Utah, Number Cruncher Statistical Systems.

Hubble, J. D., D. E. Fast, T. B. Scribner, W. R. Sharp and M. V. Johnson. 1990. Yakima/Klickitat natural production and enhancement program, annual report FY 1990. Toppenish,

Washington, Yakama Indian Nation Fish and Wildlife Resource Management. Report prepared for U. S. Department of Energy, Bonneville Power Administration, Project number 88-120.

Johnson, O. W. and 7 co-authors. 1999. Status review of coastal cutthroat trout from Washington, Oregon, and California. Seattle, National Marine Fisheries Service, NOAA Technical Memorandum NMFS–NWFSC–37.

Jordan, D. S. and B. W. Evermann. 1902. American food and game fishes. New York, Doubleday, Page & Company.

Kruse, C. G. and W. A. Hubert. 1997. Proposed standard weight (W_s) equations for interior cutthroat trout. North American Journal of Fisheries Management 17: 784-790.

Leary, R. F. 1997. Hybridization between introduced and native trout in waters of the Colville National Forest. Missoula, University of Montana Division of Biological Sciences, Wild Trout and Salmon Genetics Laboratory Report 97/3.

Leopold, L. B. 1994. A view of the river. Cambridge, Massachusetts, Harvard University Press.

Leopold, L. B., W. W. Emmett, H. L. Silvey and D. L. Rosgen, presenters. 1995. A guide to field identification of bankfull stage in the western United States. Fort Collins, Colorado, USDA Forest Service, Rocky Mountainb Forest and Range Experiment Station, Stream Systems Technology Center, 31-minute video.

McIntosh, B. A. and 6 co-authors. 1994. Historical changes in fish habitat for select river basins of eastern Oregon and Washington. Northwest Science 68, special issue: 36-53.

Murphy, B. R., D. W. Willis and T. A. Springer. 1991. The relative weight index in fisheries management: status and needs. Fisheries 16, no. 2: 30-38.

NWPPC 1989. Yakima River subbasin salmon and steelhead plan. Portland, Oregon, Northwest Power Planning Council.

Omernik, J. M. 1987. Ecoregions of the conterminous United States. Annals of the Association of American Geographers 77: 118-125.

Pater, D. E. and 7 co-authors. 1998. Ecoregions of western Washington and Oregon. Corvallis, Oregon, U. S. Environmental Protection Agency and 6 co-sponsors.

Peters, R. J., E. E. Knudsen and G. B. Pauley. 1996. The effects of fry supplementation on the emigration of salmonids from tributaries of the Clearwater River, Washington. Olympia, Western Washington Fishery Resource Office, U. S. Fish and Wildlife Service.

Phelps, S. R. 1993. Patterns of genetic diversity in Yakima River rainbow trout: initial analysis of 1990, 1991, and spring 1992 collections. Appendix A in Pearsons, T. N. and 5 co-authors. Yakima species interaction study, annual report 1992. Report prepared for U. S. Department of Energy, Bonneville Power Administration. Project 89-105.

Preiser, K., T. Friedrich and P. Renne. 1997. The use of clove oil as an anesthetic. Syracuse, New York, Iroquois Chapter, Trout Unlimited.

Proebstel, D. 1998. Unpublished report to B. K. Ringel, U. S. Fish and Wildlife Service, Leavenworth, Washington.

Proebstel, D. S. and S. M. Noble. 1994. Are “pure” native trout in the mid-Columbia River basin? Pages 177-184 in R. Barnhart, B. Shake and R. H. Hamre, editors. Wild Trout V: wild trout in the 21st Century. Yellowstone NP, UDSI–National Biological Service and others, sponsors.

Ricker, W. E. 1975. Computation and interpretation of biological statistics of fish populations. Ottawa, Fisheries Research Board of Canada Bulletin 191.

Ringel, B. K. 1996, 1997. Unpublished reports. U. S. Fish and Wildlife Service, Leavenworth, Washington.

Rinne, J. N. and M. D. Jakle. 1981. The photarium: a device for taking natural photographs of live fish. Progressive Fish-Culturist 43, no. 4: 201-204.

Sage, G. K., R. F. Leary, and F. W. Allendorf. 1992. Genetic analysis of 45 trout populations in the Yaak River drainage, Montana. Missoula, University of Montana Population Genetics Laboratory Report 92/3.

Shiozawa, D. K., J. Kudo, R. P. Evans, S. R. Woodward, and R. N. Williams. 1992. DNA extraction from preserved trout tissues. Great Basin Naturalist 52, no. 1: 29-34.

Simkins, D. G. and W. A. Hubert. 1996. Proposed revision of the standard-weight equation for rainbow trout. Journal of Freshwater Ecology 11, no. 3: 319-325.

Smith, L. J. and five co-authors. 1987. Extraction of cellular DNA from human cells and tissues fixed in ethanol. Analytical Biochemistry 160: 135-138.

Spruell, P., K. L. Knudsen, J. W. P. Smithwick, and F. W. Allendorf. 1997. Genetic monitoring of westslope cutthroat trout in the North Fork of the Clearwater River. Annual Report to the Nez Perce Tribe. Missoula, University of Montana, Division of Biological Sciences, Wild Trout and Salmon Genetics Laboratory.

Spruell, P., M. L. Bartron, N. Kanda, and F. W. Allendorf. 1999. Detection of hybrids between bull trout (*Salvelinus confluentus*) and brook trout (*Salvelinus fontinalis*) using PCR primers complementary to interspersed nuclear elements. Copeia (submitted).

Strahler, A. N. 1957. Quantitative analysis of watershed geomorphology. Transactions of the American Geophysical Union 38: 913-920.

Tripp, D. and P. McCart. 1983. Effects of different coho stocking strategies on coho and cutthroat trout production in isolated headwater streams. Ottawa, Canadian Technical Report of Fisheries and Aquatic Sciences, no. 1212.

Trotter, P. C., P. A. Bisson and B. Fransen. 1993. Status and plight of the searun cutthroat trout. Pages 203-212 in J. G. Cloud and G. H. Thorgaard, editors. Genetic conservation of salmonid fishes. New York, Plenum Press.

Varley, J. D. 1979. Record of egg shipments from Yellowstone fishes, 1914-1955. U. S. Department of the Interior, National Park Service, Yellowstone National Park. Information Paper no. 36.

Washington State Fish Commissioner. 1905, 1907….through 1919. Annual reports of the State Fish Commissioner. Olympia, Washington Department of Fisheries and Game. 18th and 19th annual reports for 1903 and 1904 through 25th and 26th annual reports for 1917 and 1918.

WDFW. 1998. Washington State salmonid stock inventory: bull trout. Olympia, Washington Department of Fish and Wildlife.

Wege, G. J. and R. O. Anderson. 1978. Relative weight (W_r): a new index of condition for largemouth bass. Pages 79-91 in G. D. Novinger and J. G. Dillard, editors. New approaches to the management of small impoundments. Bethesda, Maryland, American Fisheries Society North Central Division, Special Publication 5.

Weigel, D. E. 1997. Genetic inventory of westslope cutthroat trout in the North Fork Clearwater Basin, Idaho. Annual Report 1996. Orofino, Idaho, Nez Perce Tribe Department of Fisheries Resource Management, prepared for U. S. Department of Energy, Bonneville Power Administration, Project Number 95-016-00.

APPENDIX A

HABITAT ASSESSMENT FIELD DATA SHEET (1 of 3)

Stream Name	**Stream Code Letter**
Reach Location	**Reach Code Letter**
Investigators	
Form Completed By	**Date**

Habitat Parameter	**Optimal** (20-16)	**Suboptimal** (15-11)	**Marginal** (10-6)	**Poor** (5-1)
1. Epifaunal Substrate/ Available Cover	>50% of the substrate favorable for epifaunal colonization; mix of snags, logs, cobble, and other stable habitat.	30 to 50% mix of stable habitat elements.	10 to 30% stable habitat. Evidence of frequent disturbance and/or instability of substrate.	Less than 10% stable substrate habitat elements.
Score				
2. LWD (>4'length & **>=**12"dia.)	Minimum 4 pieces per channel width stream length, in the wetted channel.	Average of 2-3 pieces.	Average of 1 piece.	Average of less than 1 piece per channel width stream length.
Score				
3. LWD overhanging channel or within bank-ful zone	Minimum 4 per cwsl. (Count full channel-spanning piece as 1 per bank.)	Average of 2-3 pieces.	Average of 1 piece.	Average of less than 1 piece per channel width stream length.
Score (LB)				
Score(RB)				
4. Riffle	Well-developed riffle/run; riffle as wide as stream,	Riffle as wide as stream; but less than 2x width in	Riffle not as wide as stream; length <2x width;	Riffle or runs virtually non-existent; bedrock

Quality	length >=2x width; abundance of cobble.	length; abundance of cobble. Boulders and gravel present.	gravel or bedrock prevalent; some cobble present.	and/or silt/sand prevalent cobble and gravel lacking
Score				
5.Embedded-ness	Gravel, cobble, boulders less than 25% surrounded by fine sediment.	25 to 50% surrounded by fines.	50 to 75% surrounded by fines.	More than 75% surrounded by fines.
Score				
6. Pool Substrate	Mixture of materials; gravel and firm sand prevalent; roots and sub-merged veg. present.	Mixture of soft sand, mud or clay; some root mats and submerged veg. present.	All mud, clay, or sand; little or no root mats; no submerged vegetation present.	Hard-pan clay or bedrock; no root mats or vegetation.
Score				
Habitat Parameter	Optimal (20-16)	Suboptimal (15-11)	Marginal (10-6)	Poor (5-1)
7. Pool Variability	Even mixture of large-shallow, large-deep, small-shallow & small-deep pools present.	Majority of pools large-deep; very few shallow.	Shallow pools much more prevalent than deep pools.	Majority of pools small-shallow or pools absent.
Score				
8. Channel Alteration	Channelization or dredging absent; stream pattern normal.	Some channelization present, usually around road crossings; evidence of recent (last 20 yrs.) channelization absent.	Channelization may be extensive; bank hardening present; 40 to 80% of stream reach channelized and disrupted.	Banks hardened with gabion or cement. Over 80% of reach disrupted or channelized. Instream habitat greatly altered or removed entirely.
Score				
9. Sediment Deposition	Little or no enlargement of islands/point bars and <5% of stream bottom affected by fine sediment deposition.	Some new increase in bar formation, mostly from gravel and finer sediment 5-30% of bottom affected; slight deposition in pools.	Moderate deposition new gravel/finer sediments on old & new bars; 30-50% bottom affected;moderate deposition in pools prevalent.	Heavy deposition of fines increased bar dvlpment; >50% of the bottom changing frequently; pools almost absent due to deposition of fines.
Score				
10. Riffle Frequency/ Velocity-depth com-	Riffles relatively frequent distance between riffles/ stream width is <7:1. All 4 velocity-depth patterns	Riffles infrequent. Dist-ance between riffles/ stream width is 7 to 15. Only 3 of 4 velocity-	Riffles occassional; distance/width ratio is 15 to 25. Usually only 2 velocity-depth patterns	Generally all flat water or shallow riffles. distance/width ratio>25. One velocity/depth

binations	present.	depth patterns present.	present.	pattern dominant.
Score				
11. Channel flow status.	Water reaches base of both lower banks. Little substrate exposed.	Water fills>75% channel; or <25% substrate is exposed.	Water fills 25-75% of channel; riffle substrates mostly exposed.	Little water in channel; mostly present as standing pools.
Score				
12. Bank Vegetative Cover.	>90% streambank surfaces covered by native veg. Human-caused disruption minimal; almost all plants allowed to grow naturally.	70 to 90% streambank covered by native veg., but one class(tree,shrub, etc.)underrepresented. disruption evident but not affecting full plant growth	50 to 70% bank surfaces covered vy veg.; disruption obvious; patches of bare soil and/or closely cropped veg. common.	Less than 50% bank surfaces covered; high level of disruption is very high and evident.
Score (LB)				
Score (RB)				
13. Bank Stability	Evidence of erosion or bank failure minimal or absent; little potential for future problems; <5% of bank affected.	Moderately stable; infrequent, small areas of erosion, mostly healed. 5 to 30% of bank has areas of erosion.	Moderately unstable; 30 to 60% has areas of erosion; high erosion potential, exp. During flood events.	Unstable; many eroded areas; obvious bank sloughing; >60% of bank has erosional scars.
Score (LB)				
Score (RB)				
Habitat Parameter	Optimal (20-16)	Suboptimal (15-11)	Marginal (10-6)	Poor (5-1)
14. Riparian zone width.	Riparian zone >18 meters Human activities have not impacted the zone.	Width 12 to 18 meters. Human activities have impacted zone only minimally.	Width 6 to 12 meters. Human activites have noticeably,significantly impacted zone.	Width <6 meters; little or no riparian veg. Due to human activities.
Score (LB)				
Score (RB)				
Overall Total Score:				

15.Riparian zone tree maturity & composition	Evergreen Species Present:	Douglas fir W. Red Cedar W. Hemlock E. Spruce Sitka spruce Noble Fir	Evergreen Species Maturities:	% at full site potential stand height % older than 20 years but less than full site potential height % less than 20 years old

		Subalpine fir		
		P. Pine		% Old Growth
		Lodgepole		% Second Growth
		Larch		% Third or later growth
		other		
	Deciduous	Cottonwood	Deciduous	% older than 100 yrs.
	Species	Red Alder	Species	
	Present:	B L Maple	Maturities:	% older than 50 but less
		Willow		than 100 years
		Others		% older than 15 but less
				than 50 years
				% younger than 15 years

Reach Gradient:

Reach	Bedrock	Plane-Bed	Other, comment:
Channel	Cascade	Pool-Riffle	
Form.	Step-Pool	Braided	

ADDITIONAL OBSERVATIONS

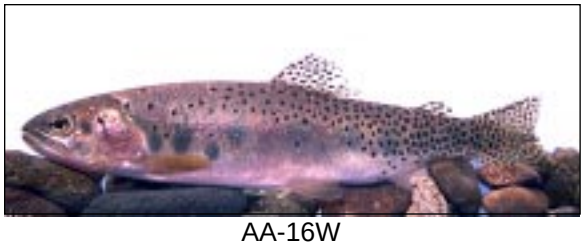
Appendix B

The Yakima River

Photo Documentation:

Native Trout Populations of the Upper Yakima Basin

North Fork Taneum Creek



AA-16W

AA-19W (hybrid)

AA-17W

AA-20W

AA-18W

AA-21W

Cabin Creek

BA-11W

BA-13W

BA-12W

BA-15W

Naneum Creek

CA-14W

CA-17W

CA-15W

CA-18W

CA-21W

Wilson Creek

DA-1W

DA-4W

DA-2W (hybrid)

DA-5W

DA-3W

DA-6W

Big Creek

EA-2W

EA-5W

EA-3W

EA-6W

EA-4W

EA-7W

South Fork Toppenish Creek

FA - 2W

FA - 4W

FA - 3W

FA - 5W

North Fork Antanum Creek

GA-1W

GA-4W

GA-2W

GA-5W

GA-3W

GA-7W

Cooke Creek:
Redband Rainbow Population Examples

HA-1R

HA-3R

HA-2R

HA-5R

HA-14R

Middle Fork Little Naches River

IA-1W

IA-15W

IA-13W

IA-16W

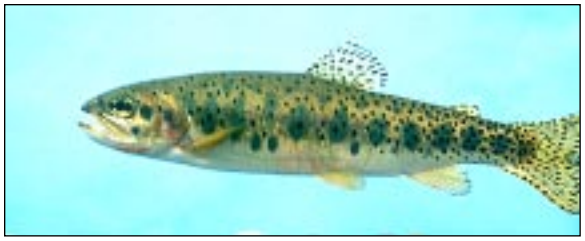
IA-14W

IA-17W

Red Rock Creek

JA-1W

JA-4W



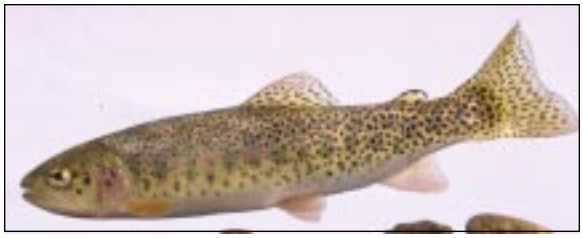
JA-2W (hybrid)

JA-5W (hybrid)

JA-3W

JA-6W

Meadow Creek



KA-1W

KA-4W

KA-2W

KA-5W

KA-3W

KA-6W